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INDUSTI CHEMISTRY

PUBLISHED BY ON E. HOWE, EDITOR

Qu of Fats

Research Laboratory E
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Prolonged heating of fats during saponification has been observed by Moore (*9*) to cause nonconjugated double bonds in fatty acids to become conjugated and thereby to absorb radiation in the ultraviolet region. Kass *et al.* (*5*) and Miller and Burr (*7*) reported that it is possible to measure quantitatively, by spectral absorption, the amount of linoleic acid in vegetable oils after heating them with a 1 to 4 solution of potassium hydroxide in ethylene glycol. The acids affected by this treatment are the unsaturated ones containing more than one double bond. In many fats the unsaturated acids consist chiefly of oleic, linoleic, and linolenic acids.

Isomerization by alkali has been studied and made the basis for the method of analysis described in this paper.

Experimental Procedure

The absorption data were obtained from measurements made with a photoelectric spectrophotometer (*2*, *8*), employing a Hilger double monochromator with crystal quartz optics. As a source of ultraviolet radiation, a Munch (*10*) hydrogen discharge tube with a fused quartz window was used. In the discussion of the results, the term "specific alpha" will be used:

$$\text{Specific } \alpha = \frac{\log_{10} \frac{I_0}{I}}{cl}$$

where α = absorption coefficient
I_0 = intensity of radiation transmitted by the solvent
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To overcome the latter difficulty, samples were heated at a temperature below the boiling point. It was found that a much weaker solution of alkaline glycol than the 1 to 4 solution previously used would serve for the isomerization. A concentration of 7.5 grams of potassium hydroxide, assaying 85 per cent potassium hydroxide, per 100 ml. of ethylene glycol, was adopted. This solution was 1.3 N with respect to potassium hydroxide. In preparation of the reagent, the alkaline glycol solution was boiled in an Erlenmeyer flask until the temperature reached 190° C. This removed most of the water, and thus it was possible to maintain a constant temperature of 180° C. while heating the samples.

The oil bath for heating the samples was maintained at 180 ± 0.1° C.

It was possible to simplify the method and to obtain consistent results by making absorption measurements directly on the soap solution, using a blank solution in the solvent cell. Thus the troublesome procedure of freeing the fatty acids and extracting them was found unnecessary.

A high dilution of the original reaction mixture is required before spectroscopic readings are taken. Ethanol is very suitable for this purpose, since the soaps are completely soluble in this solvent and do not foam when the solution is shaken. Ethanol is easily purified for optical use by distillation over potassium hydroxide and zinc dust.

INDUSTRIAL AND ENGINEERING CHEMISTRY

ANALYTICAL EDITION

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Quantitative Spectral Analysis of Fats

J. H. MITCHELL, JR., H. R. KRAYBILL, AND F. P. ZSCHEILE

Research Laboratory, American Meat Institute, University of Chicago, Chicago, Ill., and Purdue University Agricultural Experiment Station, Lafayette, Ind.

THE Kaufmann (*6*) method of analysis for oleic, linoleic, and linolenic acids in some fats is based on determinations of the iodine number, thiocyanogen number, and saturated acid content of the mixed fatty acids. The analysis for the saturated acids alone involves an iodine number determination of the separated "solid" acids and, depending upon the method used, may also involve a thiocyanogen number determination in order to correct for the unsaturated acids which are not completely separated from the solid acids.

Until recently, analyses of fats made by the Kaufmann method were in error, because theoretical values were used for the thiocyanogen numbers of linoleic and linolenic acids. Waterman *et al.* (*15*), Riemenschneider and Wheeler (*12*), and Kass *et al.* (*3, 4*), however, found the values for linoleic and linolenic acids to be empirical. Although thiocyanogen numbers are reliable when determined under carefully controlled conditions, it is desirable to have a more direct method for analysis of the linoleic and linolenic acid content of fats.

Prolonged heating of fats during saponification has been observed by Moore (*9*) to cause nonconjugated double bonds in fatty acids to become conjugated and thereby to absorb radiation in the ultraviolet region. Kass *et al.* (*5*) and Miller and Burr (*7*) reported that it is possible to measure quantitatively, by spectral absorption, the amount of linoleic acid in vegetable oils after heating them with a 1 to 4 solution of potassium hydroxide in ethylene glycol. The acids affected by this treatment are the unsaturated ones containing more than one double bond. In many fats the unsaturated acids consist chiefly of oleic, linoleic, and linolenic acids.

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where α = absorption coefficient
I_0 = intensity of radiation transmitted by the solvent
I = intensity of radiation transmitted by the solution
c = concentration of solute in grams per 1000 ml.
l = length in centimeters of solution through which the radiation passes

The first procedure tried in carrying out the isomerization was to reflux a weighed sample of fat in a 1 to 4 solution of potassium hydroxide in ethylene glycol for 25 minutes, then free the fatty acids with hydrochloric acid and extract them with ether.

Consistent results were not obtained by this method, and difficulty was experienced with emulsions in some cases. During heating of ethylene glycol and potassium hydroxide alone as a blank a substance which absorbs ultraviolet radiation is dissolved from the glass vessel or is formed from the reagents, and is extracted during the ether extraction. This, together with the fact that some oxidation of the easily oxidized conjugated double bonds may occur during the manipulation, probably accounts in part for the inability to obtain consistent results by this procedure.

Temperature is important in the rate of isomerization. Another difficulty was encountered because the boiling point of ethylene glycol–potassium hydroxide solution varies with the amount of water derived from the potassium hydroxide. The analytical reagent grade of this alkali contains about 10 per cent water. The temperature of reflux varies with the type of condenser used.

To overcome the latter difficulty, samples were heated at a temperature below the boiling point. It was found that a much weaker solution of alkaline glycol than the 1 to 4 solution previously used would serve for the isomerization. A concentration of 7.5 grams of potassium hydroxide, assaying 85 per cent potassium hydroxide, per 100 ml. of ethylene glycol, was adopted. This solution was 1.3 *N* with respect to potassium hydroxide. In preparation of the reagent, the alkaline glycol solution was boiled in an Erlenmeyer flask until the temperature reached 190° C. This removed most of the water, and thus it was possible to maintain a constant temperature of 180° C. while heating the samples.

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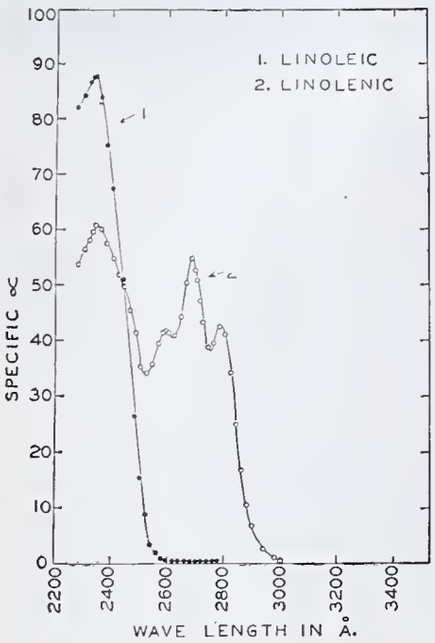

FIGURE 1. ABSORPTION SPECTRA OF POTASSIUM LINOLEATE AND POTASSIUM LINOLENATE FORMED BY ALKALI TREATMENT OF ETHYL ESTERS

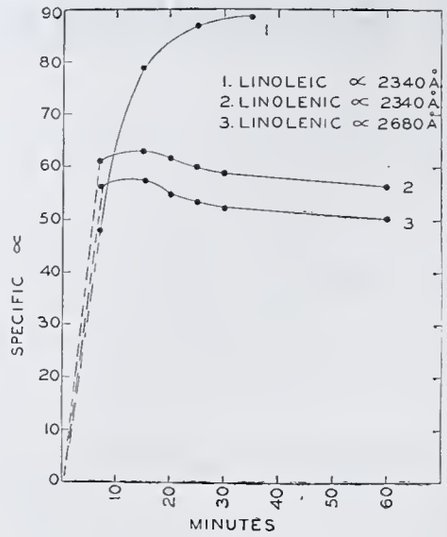

FIGURE 2. EFFECT OF TIME OF HEATING AT 180° WITH ALKALI ON SPECIFIC ABSORPTION COEFFICIENTS OF LINOLEIC AND LINOLENIC ACIDS

Only one band is present in the isomerized linoleic acid and it is due to diene conjugation. The specific alpha value at 2340 Å. is 87.1. The band with a maximum of 53.7 at 2680 Å. is due to triene conjugation, while the maximum of 60.0 at 2340 Å. in this same curve is due to diene conjugation in the isomerized linolenic acid. The reproducibility of these values is illustrated in Table I. The determinations were made at several different times and with alkaline glycol which was made up fresh each time.

In analysis, the intensity of absorption at 2680 Å. is a measure of the amount of linolenic acid present, while the intensity of absorption at 2340 Å. is a measure of both linoleic and linolenic acids. In case both acids are present, a correction is made for the absorption at 2340 Å. which is due to diene conjugation resulting from the linolenic acid.

Method

Weigh out accurately about 0.1 gram of fat or fatty acids into a small vial of the type used for iodine number determinations. Add 10 ml. of the alkaline glycol reagent, with a pipet, to a 15 × 2.5 cm. (6 inch × 1 inch) test tube and place in an oil bath at 180° C. Cover the tube with a loosely fitting glass top. The tubes should always be immersed in the bath to a constant depth. When the temperature of the reagent in the test tube has reached 180° C., drop in the vial containing the fat sample. Swirl the tube three times at 1-minute intervals to mix the fat with the glycol solution.

At the end of 25 minutes remove the tube and cool rapidly under the tap. Transfer the contents of the tube quantitatively to a 250-ml. volumetric flask, using ethanol to wash out the tube, and dilute to volume with 99 per cent ethanol.

Allow the samples to stand in a refrigerator for 5 or 6 hours, or overnight. At the end of this time, material removed from the glass by the hot alkaline solution will have precipitated. Bring the solution in the volumetric flask to room temperature and filter a portion of the solution. Make proper dilutions for absorption measurements using 99 per cent ethanol.

It is necessary to carry a blank solution, consisting of alkaline glycol, throughout the whole of the procedure, including dilutions, for use in the solvent cell.

Preparation of Standards

Linoleic and linolenic acids were prepared by bromination (*13, 14*) and debromination (*11*) of the mixed acids of corn oil and linseed oil, respectively.

The tetrabromide, after several recrystallizations from petroleum ether, had a melting point, obtained with a total immersion thermometer, of 115.5° C. The ethyl linoleate prepared by debromination of this product with zinc and 7.5 *N* anhydrous ethanolic hydrochloric acid had an iodine number of 163.8; theory 164.4.

The hexabromide, after several recrystallizations from xylene, had a melting point, obtained with a total immersion thermometer, of 185.5° C. The ethyl linolenate obtained by debromination with zinc and 7.5 *N* anhydrous ethanolic hydrochloric acid had an iodine number of 247.1; theory 248.8.

In obtaining standard absorption values for these acids, the esters were weighed and a conversion was made by a factor to obtain the equivalent weight of acid. Samples of approximately 0.1 gram were used and treated as outlined previously.

Results and Discussions

Figure 1 shows the absorption curves obtained for the potassium soaps of linoleic and linolenic acids after the alkali isomerization.

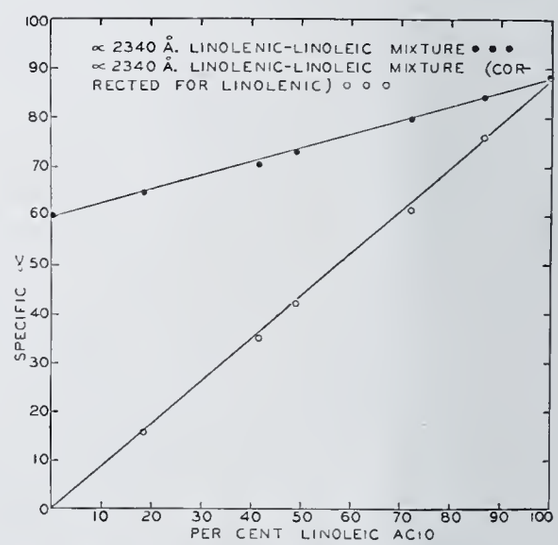

FIGURE 3. RELATIONSHIP OF SPECIFIC ALPHA VALUES AT 2340 Å. AFTER ISOMERIZATION TO PER CENT LINOLEIC ACID IN LINOLEIC-LINOLENIC ACID MIXTURE

The effect of time on the course of the reaction was studied with both linoleic and linolenic acids. The absorption rises rapidly at first (Figure 2), indicating rapid conversion of the double bonds to conjugated positions. The maximum absorption at 2340 Å and 2680 Å. is reached at the end of about 15 minutes with linolenic acid and then begins to drop gradually. The absorption of linoleic acid continues to rise slowly with

TABLE I. STANDARD VALUES FOR USE IN ANALYSIS

For Linoleic Acid (Isomerized), Specific α at 2340 Å.	For Linolenic Acid (Isomerized) Specific α at 2340 Å.	Specific α at 2680 Å.
87.1	60.1	53.7
86.9	60.1	53.6
87.2	60.1	53.6
87.1	59.7	54.0
Av. 87.1	60.0	53.7

Table II. Analyses of Linoleic-Linolenic Mixtures by Spectroscopic Method

Observed Alpha, 2680 Å.	Linolenic Found	Linolenic Added	Difference in Composition	Error	Alpha 2340 Å.[a]	Linoleic Found	Linoleic Added	Difference in Composition	Error
L./g. cm.	%	%	%	%	*L./g. cm.*	%	%	%	%
7.42	13.8	13.4	+0.4	+2.99	76.1	87.4	86.6	+0.8	+0.92
14.8	27.6	28.0	−0.4	−1.43	62.2	71.4	72.0	−0.6	−0.83
27.4	51.0	51.1	−0.1	−0.20	42.2	48.4	48.9	−0.5	−1.02
31.8	59.2	58.3	+0.9	+1.54	35.0	40.2	41.7	−1.5	−3.60
44.0	81.9	81.5	+0.4	+0.49	15.7	18.0	18.5	−0.5	−2.78
				Av. ±1.33					Av. ±1.83

[a] Corrected for the absorption of linolenic acid at 2340 Å.

Table III. Comparison of Analyses by Spectroscopic and Kaufmann Methods

Sample	Iodine No. (Glyceride) Wijs Method	Linolenic Acid S	Linolenic Acid K	Linoleic Acid S	Linoleic Acid K	Oleic Acid S[a]	Oleic Acid K	Saturated Acids S[b]	Saturated Acids K
		%	%	%	%	%	%	%	%
Linseed	179.7	47.3	46.8	18.2	22.4	28.6	22.8	5.9	8.5
Soybean 1	136.5	9.88	8.95	53.2	53.7	21.6	23.3	15.4	14.0
Soybean 2	134.3	7.47	8.03	54.7	53.5	23.4	23.9	14.5	14.5
Soybean 3	130.0	5.23	6.23	56.2	52.0	21.8	27.3	16.8	14.5
Cottonseed	110.1	0.00	0.00	54.8	54.2	17.8	18.9	27.4	26.9

[a] $\% \text{ oleic acid} = \dfrac{\text{I No. of mixed acids} - (\%\text{ linolenic})(\text{I No. of linolenic}) - (\%\text{ linoleic}) \times (\text{I No. of linoleic})}{\text{I No. of oleic}}$.

[b] % saturated acids = 100% − % unsaturated acids.

time. The change is not critical at the end of 25 minutes, and this period was used in the method of analysis.

Known mixtures of linoleic and linolenic acids were made up and analyzed by the spectroscopic method. The alpha values at 2340 Å. fall on a straight line (Figure 3) when plotted against the percentage of linoleic acid. The top line represents the total alpha values at 2340 Å. due to both linoleic and linolenic acids together, while the lower line represents the alpha values after correction for the linolenic acid which was determined by the absorption band at 2680 Å.

The alpha values at 2680 Å. for various mixtures of linoleic and linolenic acids have been plotted (Figure 4) against the percentage of linolenic acid. These values fall on the same straight line with those obtained for a mixture of stearic and linolenic acids. It is obvious that neither the saturated acids nor linoleic acid interfere with the determination of linolenic acid.

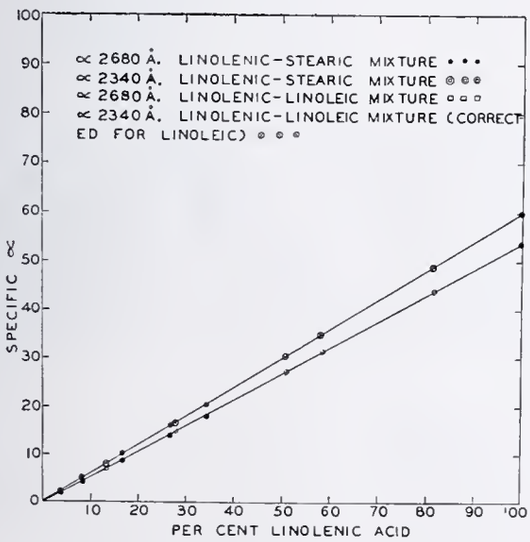

Figure 4. Relationship of Specific Alpha at 2340 Å. and 2680 Å. to Per Cent Linolenic Acid in Linolenic-Linoleic and Linolenic-Stearic Mixtures

The alpha values at 2340 Å., after correction for the absorption due to linoleic acid, have been plotted and fall on the same straight line with those obtained for a mixture of stearic and linolenic acids.

The actual percentages of linoleic and linolenic acids which were added to the mixtures and the percentages which were determined by the spectroscopic method are compared in Table II. Linolenic acid was determined with a percentage error of ±1.33 and linoleic acid was determined with a percentage error of ±1.83.

For the analysis of a fat (which consists chiefly of saturated acids, oleic, linoleic, and linolenic acids) by the spectroscopic method it is necessary to know only the iodine number of the oil before isomerization in addition to the amount of linoleic and linolenic acids in order to calculate the percentage of oleic acid. The saturated acids are then obtained by difference. Several samples of soybean oil, linseed oil, and cottonseed oil were analyzed by both the Kaufmann and the spectroscopic methods. The results are compared in Table III.

The saturated acids in the Kaufmann method were determined by crystallization from acetone at −40° C. (*1*).

Conclusions

A spectroscopic method is described for direct determination of the linoleic and linolenic acid content of a fat. These acids can be determined very simply and as accurately as standard values for the pure acids can be obtained, when the fats do not contain other acids with two or more double bonds. Making use of the iodine number, the oleic acid content can be obtained; the saturated acids are then obtained by difference. Thus an analysis can be obtained on many fats (those containing chiefly saturated acids, oleic, linoleic, and linolenic acids) with as little as 0.2 gram of sample.

The method is more rapid than the Kaufmann method and involves fewer determinations.

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330715

Analysis for Potassium by Its Natural Radioactivity

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PRESENT-day knowledge of nuclear physics suggests that radioactive isotopes of all the elements probably existed when the earth was formed. However, only members of the uranium-radium, uranium-actinium, and thorium series and the elements potassium, rubidium, and samarium still show appreciable natural radioactivity. (There is also some evidence that lutecium and neodymium have weak activity, *8*.) The survival of these is explained by the fact that they have half-lives of the order of 10^9 years, which is so long that they exist yet in detectable quantities. All the rest of the radio-isotopes originally present must have had comparatively short life periods and consequently have completely decayed during the ages, or else they decay so slowly that they cannot be detected readily. Evidence of this is the fact that the three hundred or more radio-isotopes already produced artificially have half-life periods ranging from a few seconds to only a few years. Only two, B^{10} and C^{14}, have half-lives greater than 10^3 years, and even such periods seem short in comparison with geologic time.

One of the pieces of evidence which proved that potassium was naturally radioactive was the observation that the activity of a given sample is directly proportional to its potassium content (*6*). Apparently, however, no attempt was made to apply this to the quantitative analysis of materials for their potassium content. [Since this work was completed, Fenn, Bale, and Mullins (*5*) have described determinations of the radioactivity of potassium from bone ash with a dipping counter tube. However, these authors did not attempt to develop a procedure for routine potassium analyses.] Such an application, were it possible to carry out simply, would appear to be of great value, since the determination of potassium in the presence of other alkalies and certain anions is a difficult and time-consuming procedure. Accordingly, the work recorded here was undertaken in order to establish a rapid method of analysis for potassium, based on its natural radioactivity, which would be free from interference by other elements and would lend itself to routine operation. The experiments described here show the practical nature of this method, and indicate as well the various factors affecting such a determination.

Three of the questions which bear on the subject may be briefly disposed of here. (1) One concerns the possible interference by the other natural radio-elements. Of these rubidium causes no concern, for it is very rare in occurrence and emits a soft beta-ray, only one third as energetic as that of potassium, and no gamma-ray. Samarium too is very rare, and furthermore, since it is an alpha-emitter, it would not be counted in the tube employed. However, the members of the uranium or of the thorium series would, if present, invalidate the potassium analysis by their radioactivity. Since these elements are met only rarely, they do not seriously restrict the application of the method, and, even if present, they can be removed comparatively easily by standard procedures.

(2) A second factor has to do with the natural rate of decay of the potassium. The well-known law for radioactive disintegration is

$$N = N_0 e^{-\lambda t}$$

where N is the number of radioactive atoms at time t, N_0 is the number initially present, and λ is the decay constant. For potassium $\lambda = 1.56 \times 10^{-17}$ sec.$^{-1}$ which in terms of half-life is 1.4×10^9 years (*1*). Consequently, no detectable change would occur in activity of the potassium in the time required for analysis.

(3) The naturally occurring activity of potassium is due to the isotope of mass number 40 (*13*). Isotopes 39 and 41 are stable, and the abundance of masses 39, 40, and 41 are 93.3, 0.012, and 6.7 per cent, respectively (*2*, *9*). The analytical method developed here depends, consequently, on the assumption that no isotopic separation occurred during any of the ordinary processes to which the sample may have been subjected. This assumption seems reasonable, for the appreciable separation of isotopes of such high atomic weight by the usual chemical and physical procedures is negligible. Moreover, the constancy of the ratio of the potassium isotopes in mineral matter has been fairly well established. Smythe (*12*) and Brewer (*3*) have shown that there is no variation either in the K^{39}/K^{40} or in the K^{39}/K^{41} ratio in various rocks, in sea water, and in commercial potassium. A slight separation seems to be discernable in plant and animal specimens, but the amount and the direction of the isotopic shift are variable (*4*, *5*). Such small changes would be completely unimportant for the purposes at hand.

Experimental

The apparatus used for the preliminary experimental work consisted of a small Geiger counter tube in a lead shield, an amplifying and recording circuit, and a glass cell for the solutions. The Geiger tube was of thin-walled glass about 1.5 cm. in outside diameter and 15 cm. long, silvered on the inside wall. It was filled to about 100-mm. pressure with a "fast" counting gas consisting of 90 per cent argon and 10 per cent alcohol. The

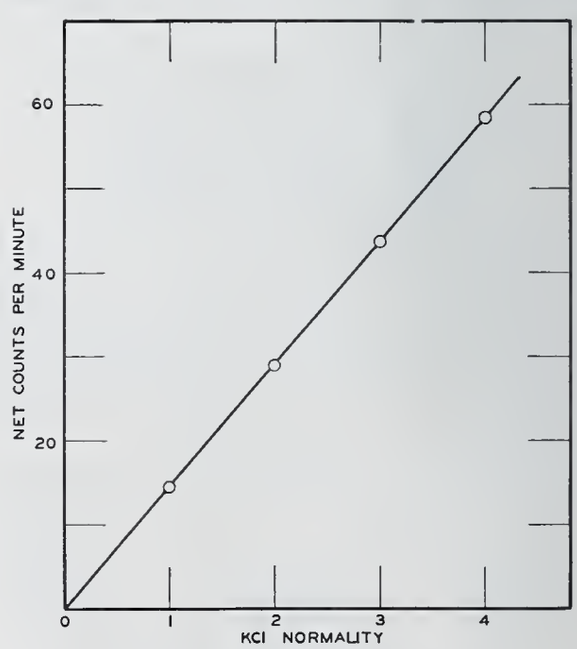

FIGURE 1. COUNTING RATE *vs.* POTASSIUM CONCENTRATION
Small counter tube

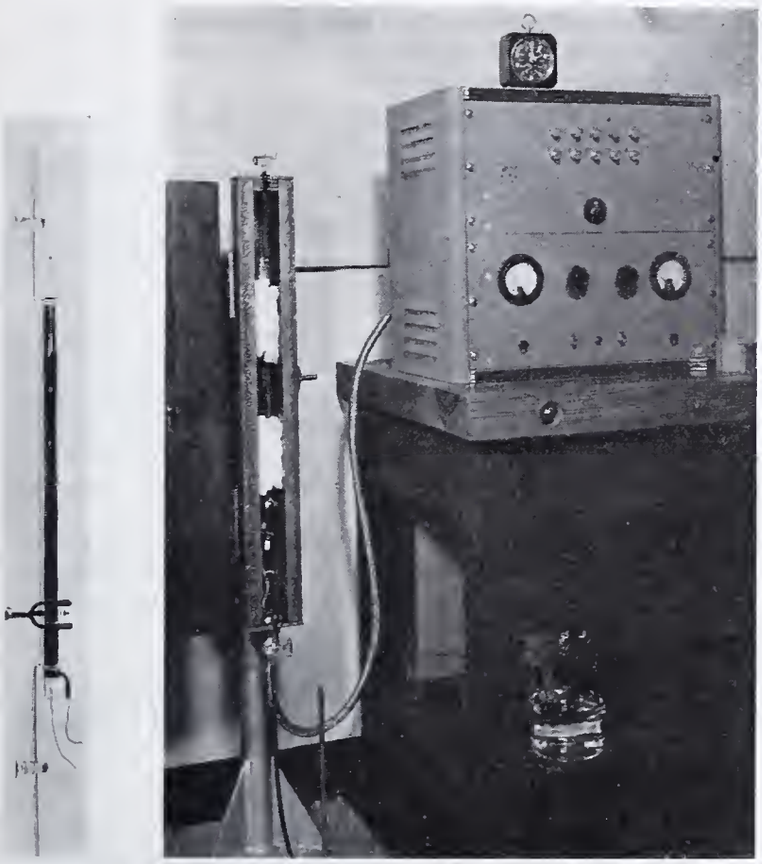

FIGURE 2
(*Left*) Large counter tube. (*Right*) Large counter tube and circuit

TABLE I. COMPARISON OF KNOWN AND CALCULATED POTASSIUM CONCENTRATIONS

Potassium Salt	Added Salt Kind	Added Salt Normality	Net Count per Minute	Potassium Normality Known	Potassium Normality Calculated
KCl	...	...	14.3	1.0	0.98
KCl	...	...	29.1	2.0	1.98
KCl	...	...	44.0	3.0	3.00
KCl	...	...	58.4	4.0	3.96
KCl	NaCl	3.0	14.8	1.0	1.01
KCl	NaCl	1.0	43.5	3.0	2.97
$K_3Fe(CN)_6$	...	...	14.1	1.0	0.96
$K_3Fe(CN)_6$	...	...	44.1	3.0	3.01
K_2CO_3	...	...	12.1	1.0	0.87
K_2CO_3	...	...	44.3	3.0	3.03
KCl	NaCl $Fe(NH_4)(SO_4)_2$ $CaCl_2$ NH_4Cl	0.68 0.008 0.18 0.38	17.0	1.07	1.16

amplifier and scaling circuit was of conventional type with a simple circuit as the first stage. The cell for the solutions was a thin-walled annular glass vessel which fitted closely around the counter tube, and held about 20 cc. of solution.

The procedure adopted was to prepare solutions of known concentrations of various potassium salts, alone and mixed with other salts, and to make a 2-hour count on each. Background counts were taken for the same length of time with distilled water in the cell. (The background throughout these experiments remained very constant and was about 25 counts per minute.)

The data obtained for all the solutions measured are assembled in Table I. Examination of the data for 1, 2, 3, and 4 *N* potassium chloride solution indicates that the net count was directly proportional to the concentration. This is shown graphically in Figure 1, in which the best straight line is drawn through the points. For all the other solutions, therefore, the normality corresponding to an observed count could be read directly from the graph; these are the so-called "calculated normality" figures recorded in the last column of Table I. That the method has practical possibilities is obvious from the agreement of the known and calculated values.

The observation that the count was directly proportional to the concentration of potassium was to be expected from the original work on the activity of potassium. Of great importance for the purpose at hand was the fact brought out in Table I, that the nature of the accompanying anion or of added salts had such a small effect on the counting rate that no interference was apparent with this apparatus. While these results, therefore, showed the feasibility of performing potassium analyses by a radioactive method, the procedure was unsatisfactory because of the low sensitivity of the counter tube. Even for rather concentrated solutions, the observed count was only 15 to 60 counts per minute above the background. Consequently, excessively long times were required for any reasonable accuracy and samples of low concentration could not be determined successfully.

In order to show how sensitivity, accuracy, and time of analysis are interrelated, a short discussion may be inserted at this point.

The disintegration of a radioactive substance is a random process subject to statistical laws which can be deduced from probability considerations. Thus, in order to measure the activity of a material with absolute accuracy, a count of the number of particles disintegrating in an infinitely long time would be required. However, it may be shown that the probable error in a single set of counts for a given time interval is 0.67 of the square root of the number of counts observed. For example, if 10,000 counts have been made, the probable error in this measurement is $0.67\sqrt{10{,}000}$ or 67 counts, and the relative accuracy would be ± 0.67 per cent. For a relative accuracy of ± 1 per cent it would be necessary to count approximately 4500 emissions. The following table indicates the relationship between the probable error and minimum number of counts required for the stated accuracy:

Probable Error, %	Total Count Required
10	45
5	180
3	450
1	4,500
0.5	18,000
0.1	450,000

The presence of the background count introduces an additional source of error. Whenever the number of counts from a sample is so small that it approaches the background count, it is then necessary to count from five to ten times as many disintegrations to obtain a desired precision for the measurement. A further discussion of these points may be obtained from Rasetti (*11*) or from Strong (*14*).

The foregoing paragraph makes it clear that a more sensitive tube would extend the over-all accuracy of the method or decrease the time required for performing an analysis of a given accuracy. Inasmuch as this sensitivity of a counter tube is a function of its geometry and size, a new tube was designed to accomplish this.

Although similar to the first type in that it was of thin-walled glass silvered on the inside, it was considerably larger and had an outer jacket built around the counter tube proper. The annular space so formed held the solution to be counted, and could be filled by sucking liquid up through a lower inlet. With this design only one thin glass wall was interposed between the solution and counter interior. Tubes similar in principle but smaller in size have already been described (*7*, *10*). The tube is shown in Figure 2; on the right it is shown in place in its lead shield and with the amplifying circuit connected. The diameter of the inner tube is about 2.4 cm., the outer tube or jacket is 3.5 cm., and the spacing between the walls of jacket and inner tube is about 4 mm. The jacketed portion of the tube is about 45 cm. long, and the volume of the jacketed space is about 200 cc. The counter gas used is the usual 90-10 argon-alcohol mixture at a total pressure of 100 mm., and the tube is operated on the con-

ventional simple circuit. It showed excellent counting characteristics under these conditions with a threshold of about 900 volts and a plateau 100 to 140 volts long.

A calibration of two different tubes was run with standard potassium chloride solutions. Twenty-minute counts were made, and background was counted with distilled water in place of the potassium chloride solution.

The background count in the absence of distilled water is always higher than with water present. This effect was noted with all the tubes tried, and the cause is not explained. Possibly the water provides additional shielding from external radiation, perhaps from the lead itself It does not seem to be due to a natural radioactivity of the Pyrex, for counts of samples of Pyrex have shown no appreciable activity. In any event, the water is sufficiently dense to screen out all such external radiation, as a solution of zinc chloride of density 1.5 gave the same count as distilled water. Exposure to sunlight increases the counting rate, and consequently, if no lead housing is available, the tube at least should be covered with a black cloth.

The results are shown in Figure 3, in which counting rate is plotted against the normality of the potassium chloride. The background count has been included in these calibration curves in order to indicate the magnitude of the counts actually recorded.

The total sensitivity of the method was increased about 30-fold by using the larger tube, for the net count of 1 N solutions in the large counter was about 400 counts per minute as compared to 14 for the small one. Clearly, solutions as dilute as 0.1 to 0.05 N may be analyzed with fair accuracy by making rather long counts. For concentrations of 1 N or higher, the precision for a 20-minute count is about 0.01 N, as was shown by analyzing two unknown solutions:

	Potassium Normality	
	Known	By radioactivity
Unknown 1	2.274	2.26 ± 0.01
Unknown 2	1.186	1.18 ± 0.01

A closer examination of Figure 3 reveals the fact that for both new tubes the counting rate is not quite proportional to the concentration, but falls off with increasing potassium content. Such an effect was not observed with the smaller tubes, since their sensitivity was too low. The effect was ascribed

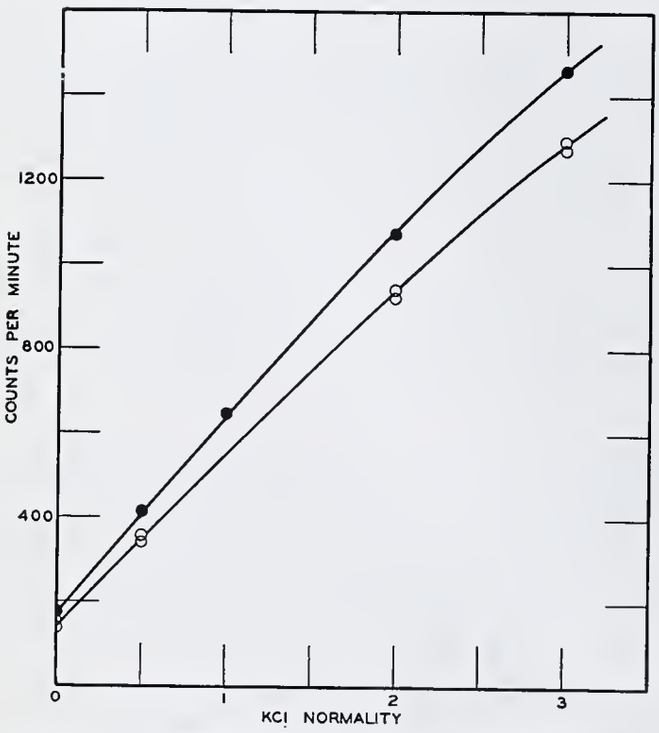

FIGURE 3. COUNTING RATE *vs.* POTASSIUM CONCENTRATION

● Large counter tube B ○ Large counter tube A

to the self-absorption of the radiation resulting from the increase in density of the solution with concentration, and this point was investigated further.

1 N solutions of potassium chloride were made up with sucrose or zinc chloride added to increase the density, and the same was done with 1 N potassium ferricyanide solutions. Using counter A, duplicate counts of 20 minutes each were made on each solution; in addition, the density of each solution was determined with a pycnometer. The results are shown in Figure 4, in which the net count per minute—i. e., background subtracted—is plotted against the observed density on semi-log paper.

The decrease in count brought about by the increase in density is obvious—for example, the counting rate was lowered about 10 per cent by an increase in density from 1.03 to 1.30. It is evident, therefore, that with these sensitive tubes a correction for the density effect is required if the density of the "unknown solution" varies markedly from that used in the calibration.

The method of representation of the measurements given in Figure 4 was chosen because the data fit an excellent straight line. The equation of this line, is, of course, of the form

$$\log (c/m) = k - (a)(d) \qquad (2)$$

where (c/m) is the counting rate, d is the density, and k and a are empirical constants. On evaluation of k and a, Equation 2 becomes:

$$\log (c/m) = 2.8525 - 0.234\, d \qquad (2)$$

This equation applies for 1 N solutions for the particular counter tube at hand. To extend it to include any normality of solution, the term log N is merely included, so that the equation reads

$$\log (c/m) = 2.8525 - 0.234\, d + \log N \qquad (3)$$

Now this equation not only fits the data of Figure 4 but also reproduces the calibration curve of counter tube A in Figure 3 within a precision of ±1 per cent. The fact that the numerical constants as obtained from the count-density curve of Figure 4 for 1 N solutions are applicable to potassium chloride solutions of any normality constitutes proof that the density is the factor responsible for the falling off of the counting rate with increased concentration, as observed in Figure 3.

A general equation of the form of (3)

$$\log (c/m) = k - (a)(d) + \log N \qquad (4)$$

applies for any counter tube, but the constants vary from tube to tube. For example, for tube B the equation as determined from the calibration curve in Figure 3 is

$$\log (c/m) = 3.1555 - 0.463\, d + \log N \qquad (5)$$

The value of k should clearly vary from counter tube to counter tube, for in effect k is the logarithm of the count which would be given by a 1 N solution of potassium at zero density. This must naturally depend on the length and wall thickness of the jacketed portion of the counter. The variation in the constant a from tube to tube may probably be due to the fact that the radiation from potassium is not homogeneous for both beta- and gamma-rays are effective in firing the counter, and furthermore, for potassium the beta-rays themselves show two different energy groups. The variation in thickness of the layer of solution from tube to tube may also play a role. It is an interesting point that an apparent half-thickness of about 1.4 grams per sq. cm. may be calculated from the slope of the curve of Figure 4. This lies between the value for the gamma-rays (about 5 grams per sq. cm.) and the beta-rays (about 0.03 gram per sq. cm.), and would suggest that the radiation actually entering the counter consisted of about 75 per cent beta-rays and 25 per cent gamma-rays, with the wall thickness of the tube about 0.2 to 0.4 mm.

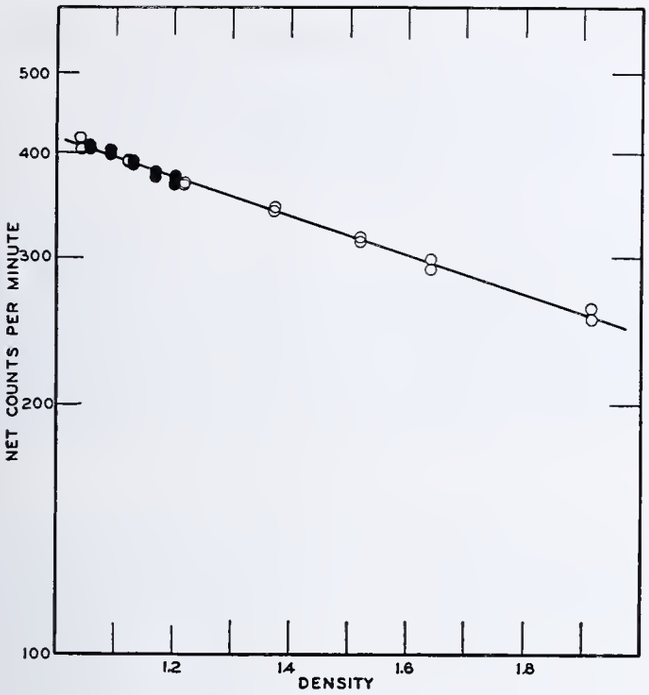

FIGURE 4. EFFECT OF DENSITY OF SOLUTION ON COUNTING RATE

● 1 N KCl + $ZnCl_2$ ○ 1 N $K_3Fe(CN)_6$ + sucrose

To make a potassium determination by the radioactive method described here, the counter tube to be used must first be calibrated. This can be done with only two solutions of potassium, say 1 N and 3 N potassium chloride. The observed counts per minute and the known densities of the calibrating solutions are substituted in Equation 4 and the constants k and a evaluated. The empirical relation between count, density, and normality is thus obtained for the counter at hand. For any unknown solutions, the counting rate and the density must be measured, the values substituted in the equation and the normality then calculated.

The above method of correcting for the effect of density is applicable when the element in the salt mainly responsible for the high density is of atomic number below zinc (Zn = 30). The density correction necessary if elements of higher atomic number are present may be as much as 50 per cent greater and seems to depend on the element. For example, in trials with potassium nitrate solutions with barium chloride or with lead nitrate added to make the density 1.33, the observed count was 94 and 90 per cent, respectively, of the count obtained when zinc chloride was used to bring the density of the potassium nitrate solution to the same value. In general, compounds with elements of such high atomic number will not be found in amounts sufficient to contribute predominantly to the density of the solution, so that the correction determined as previously described may be used safely. However, if salts of heavy metals are present in high concentration, these had best be removed before the counting of the potassium solution is undertaken. As an alternative procedure, the counter tube may be calibrated with potassium solutions increased in density by addition of the salt present in the unknowns.

On the basis of this work, an apparatus was installed for routine use in a control analytical laboratory and has been in operation for about 6 months. In that time, many analyses have been made which could have been carried out by the usual procedures only with far greater expenditure of time and labor.

Conclusions

The results presented in this report indicate that a rapid and comparatively simple determination of potassium can be made by taking advantage of its natural radioactivity. Obviously, the type of equipment used in making these analyses may be applied with advantage for work with other radioisotopes, whether natural or artificial. The size and design of the tubes employed naturally are not restricted to those described here, but may be altered to meet particular conditions. Once the apparatus has been set up, a determination requires 10 minutes to 2 or 3 hours, depending on the sensitivity of the counter, the potassium concentration, and the accuracy desired. The outfit itself is not too complicated and can be assembled at a total cost of about $450. Operation is simple, and maintenance of the apparatus is negligible, although the circuit may require adjustment occasionally. The life of the counter tube is indefinite, but usually the good counting characteristics, if lost, can be restored by pumping and refilling the tube with counter gas. The advantages of the method are obviously such as to recommend it for general use.

Acknowledgment

It is a great pleasure to acknowledge the help and advice of J. R. Dunning of Columbia University, J. W. Kennedy of the University of California, Berkeley, and R. C. Raymond of the Massachusetts Institute of Technology, in connection with the design of the counter circuit and of the tubes.

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Suspension of Glass Thermometers

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THE glass ring provided at the top of glass thermometers for suspension from a hook, wire, or string often breaks off in handling because of its rigidity. If the thermometer itself is still in working order, it is usually suspended through a rubber stopper or cork held in place by means of a buret clamp.

A better method is to fit a short piece of rubber tubing snugly over the upper end of the thermometer, so that about an inch of the tubing extends freely at the top. A small hole is cut in the end of the tubing by bending 0.25 inch of the top down sharply and making a very short lengthwise cut at the bend with a pair of scissors.

The thermometer can then be suspended freely in the usual manner. As a matter of fact, new thermometers may be thus provided. In this manner, the danger of contaminating a material with spilled mercury from a broken thermometer is considerably lessened.

Polarographic Determination of Manganese as Tri-dihydrogen Pyrophosphatomanganiate

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A procedure is presented by which manganese is quantitatively oxidized with lead dioxide to the trivalent state in a medium which contains a large excess of pyrophosphate at a pH smaller than 4. The concentration of the manganic manganese in the complex is determined polarographically.

Evidence is given that the violet complex ion is tri-dihydrogen pyrophosphatomanganiate.

THE present paper describes a simple procedure in which manganese is oxidized with the aid of lead dioxide to the manganic complex in the presence of a large excess of potassium pyrophosphate at a pH between 2.0 and 4.0. The concentration of manganic complex is determined with the aid of the dropping mercury electrode. Evidence that the complex has the composition tri-dihydrogen pyrophosphatomanganiate is given.

Manganous ion is reduced at the dropping mercury electrode, producing a well-defined diffusion current in neutral alkali chloride as supporting electrolyte (*14*). The half-wave potential is −1.51 volts *vs.* the saturated calomel electrode, so the presence of an excess of several metal ions such as ferric, ferrous, cobaltous, nickel, zinc, and copper interferes with the polarographic determination of divalent manganese. Furthermore, the hydrogen wave interferes in acidic solution. The permanganiate ion produces a poorly defined wave starting at zero applied e. m. f. Stackelberg *et al.* (*12*) concluded that this wave was not suitable for analytical purposes. Verdier (*14*) found that in an alkaline tartrate solution, a well-defined anodic wave was obtained at −0.4 volt *vs.* the saturated calomel electrode. The manganous ion is apparently oxidized to the trivalent state. A large excess of ferrous iron interferes in this determination. Tri-dihydrogen pyrophosphatomanganiate has the advantage that the manganese is reduced from the tri- to the divalent state at positive potentials *vs.* the saturated calomel electrode. As the diffusion current can be measured at +0.1 volt to +0.15 volt *vs.* saturated calomel electrode, it is not necessary to remove dissolved oxygen. A large excess of ions such as ferric, zinc, copper, cobalt, and nickel may be present. Chromium, vanadium, and cerium interfere, since these metals are oxidized to chromate, vanadate, and ceric, respectively, and are also reduced at positive potentials.

Trivalent manganese has been employed in several analytical methods for determining manganese.

Heczko (*7*) oxidized manganese to the trivalent state with freshly prepared perphosphoric acid in a solution of phosphoric acid and sulfuric acid and titrated the trivalent manganese iodometrically. Lang (*10*) oxidized manganese with potassium dichromate in a solution of metaphosphoric acid. After reducing the excess dichromate with sodium arsenite solution, he titrated the trivalent manganese with standard ferrous sulfate solution using diphenylamine as the indicator. Hirano (*8*) estimated manganese colorimetrically by forming the trivalent complex in a solution of sulfuric acid and phosphoric acid. Tomula and Aho (*13*) estimated manganese colorimetrically by forming a pyrophosphatomangani acid complex in a mixture of pyrophosphoric and sulfuric acids. They employed potassium bromate as the oxidizing agent and removed the liberated bromine through the formation of the slightly dissociated compound, bromine cyanide. This procedure has the disadvantage of the danger associated with the use of acidified cyanide solutions.

Experimental

The manual apparatus described in previous publications was used. Since the complex slowly oxidizes mercury, a saturated calomel anode was employed as outside electrode. A few polarograms were made using the automatic Heyrovský polarograph. The pH measurements were made with the aid of a Beckman glass electrode pH meter. A thermostatically controlled water bath was used to maintain a constant temperature of 25.0 ± 0.02° C.

Analytical reagents were employed. A stock solution of 0.1 *M* manganous sulfate was prepared by dissolving 22.306 grams of manganous sulfate tetrahydrate in water containing 10 ml. of approximately 1 *N* sulfuric acid and diluting to exactly 1 liter. The molarity was checked by a gravimetric determination in which the manganese was precipitated as manganous ammonium phosphate and weighed as manganous pyrophosphate. The molarity was found to be correct.

Source of Pyrophosphates

Although orthophosphoric acid may be converted to pyrophosphoric acid if heated at 215° C., large amounts of silica are dissolved if the conversion is performed in a Pyrex container. Platinum is also slowly attacked. Furthermore, concentrated pyrophosphoric acid is variable in composition (*5*) and difficult to handle because of its viscosity. Its aqueous solution is unstable, being completely hydrolyzed in a few days.

The authors investigated the use of a mixture of alkali pyrophosphates and strong mineral acids as the source of the dihydrogen pyrophosphate ion. Sodium pyrophosphate is only moderately soluble, 3.16 grams of the anhydrous salt dissolving in 100 ml. of water at 0° C. and 40.26 grams at 100° C. Potassium pyrophosphate is extremely soluble and is therefore more suitable for the preparation of a concentrated stock solution. Such a solution is stable for a long time if not acidified (*6*). The potassium pyrophosphate used in the following experiments was prepared by heating anhydrous dipotassium hydrogen phosphate in a furnace at from 500° to 700° C. for 3 hours.

Sodium pyrophosphate may be used instead of potassium pyrophosphate. In this case it is necessary to weigh out the salt for each determination.

Sulfuric and nitric acids were found satisfactory for the regulation of the acidity. Nitric acid is preferable if an appreciable amount of sulfate is present in the sample, since potassium sulfate, which is only moderately soluble, may precipitate.

Selection of an Oxidizing Agent

Lead dioxide was found to be a satisfactory oxidizing agent. It rapidly oxidized manganous ion to a violet-colored complex in a solution 0.4 *M* in pyrophosphates having a pH of 4 or less. The excess lead dioxide was removed by filtering through a retentive filter paper, such as Whatman No. 42. By means of titrations with standard ferrous sulfate solution it was shown that the oxidation was quantitative. It was

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experimentally found that 1 gram of lead dioxide could oxidize 388 mg. of manganous ion to the trivalent complex ion. This is close to the stoichiometric amount—namely, 460 mg. Practically, a large excess of lead dioxide, approximately 1 gram for each 50 mg. of manganese in the solution was used to hasten and ensure quantitative oxidation. It was found that the oxidation of a 0.01 *M* solution of manganous ion was complete after 3 minutes of shaking with this excess of lead dioxide if the pH was smaller than about 4.0 and a large excess of pyrophosphate was present. Intermittent shaking for 10 minutes resulted in quantitative oxidation of manganese in solutions as concentrated as 0.02 *M* in manganous ion. Variable concentrations of plumbous lead remained in solution, presumably as a dihydrogen pyrophosphato complex, even when appreciable concentrations of sulfates were present.

Suppression of the Maximum

In the absence of capillary-active substances a large maximum in the current-voltage curve of the manganic complex was obtained. Furthermore, the galvanometer oscillations were irregular at potentials more negative than the region (+0.1 to +0.3 volt *vs.* S. C. E.) in which the maximum occurred. These disturbances persisted in the presence of various agents generally used for the elimination of maxima. Gelatin, starch, soap, tylose, camphor, thymol, methyl orange, tropeolin 00, methyl red, and methylene blue were unsatisfactory in the suppression of the maximum.

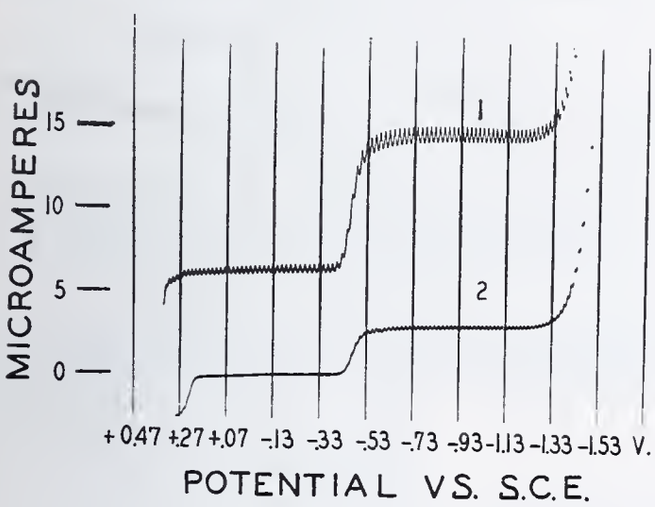


FIGURE 1. POLAROGRAM

1. 5 millimolar tri-dihydrogenpyrophosphatomanganiate, 0.4 *M* sodium pyrophosphate, 0.8–0.9 *M* nitric acid, 0.1 per cent in gum arabic, pH = 2.0; oxidized with lead dioxide; hydrogen passed through solution 15 minutes
2. Like 1 except no manganese present

It was found that if the solution was 0.02 per cent in agar the galvanometer oscillations became regular and diffusion current measurements could be made at potentials more negative than +0.16 volt (S. C. E.) using the manual polarograph. Many of the quantitative measurements were made with the manual apparatus, using agar to suppress the maximum. However, when polarographic determinations were attempted using the automatic Heyrovský polarograph, agar was found unsatisfactory. Temporary irregularities, which did not interfere when the manual instrument was used, were frequently recorded in the polarogram.

It was then found that if the final solution was 0.04 per cent in peptone (Merck's, dried, from meat) excellent waves were obtained with either the manual apparatus or the polarograph. It was necessary to add the peptone prior to the oxidation by lead dioxide. If added after the oxidation, a small amount of the complex was reduced.

After the experimental work was concluded the authors found that 0.1 per cent gum arabic is just as effective as peptone. It is stable for a long time and does not decompose the complex if added after the oxidation.

Within experimental error, the same diffusion current per millimolar concentration of the manganic complex was obtained whether 0.05 to 0.2 per cent agar, 0.02 to 0.1 per cent peptone, or 0.1 per cent gum arabic was used to eliminate the maximum.

The procedure followed in the preliminary experiments consisted of preparing solutions containing manganous ion and various concentrations of pyrophosphate at various pH values and in the presence of various maximum suppressors. The manganese was oxidized by shaking intermittently with lead dioxide for 5 to 10 minutes and the solution was filtered. Current voltage measurements were made using a portion of the filtrate. The removal of air is not necessary, since the diffusion current of the manganic complex is obtained before the oxygen wave starts.

Figure 1 illustrates the characteristics of the wave.

The solution used to obtain curve 1 was 0.005 *M* in manganic manganese, 0.4 *M* in sodium pyrophosphate, 0.8 to 0.9 *M* in nitric acid, and contained 0.1 per cent gum arabic. The pH was 2.00. The solution used to obtain curve 2 contained the same constituents, except that no manganese was added. The solutions were treated with lead dioxide as explained above. Tank hydrogen was passed through the solution in the cell for 15 minutes to remove most of the oxygen. In curve 2 the current from +0.2 to −0.4 volt (S. C. E.) was the residual or condenser current and was practically zero at −0.1 volt (S. C. E.). The anodic wave just beyond +0.25 volt is due to the dissolution of mercury. This wave begins at a lower positive potential than in the corresponding polarograms in Figures 2 and 3, owing to the presence of chloride ion introduced by prolonged contact with the saturated calomel anode. The wave beginning at −0.4 volt (S. C. E.) is due to the reduction of the plumbous lead introduced as a result of shaking with lead dioxide. The wave starting at −1.3 volt (S. C. E.) is due to the reduction of hydrogen ions. In curve 1, the wave beginning at about +0.3 volt is due to the reduction of the trivalent manganic complex to the divalent state. The second wave for the reduction of plumbous lead is larger than in curve 2 as a consequence of the reduction of more lead dioxide in the oxidation process. The true diffusion current is the difference between the magnitude of the apparent diffusion current measured and the residual current measured at the same potential, using a blank solution containing no manganese or a portion of the original solution before treatment with lead dioxide.

The half-wave potential of the wave due to the reduction of the manganic complex is obviously not a function of the standard potential of the manganous complex–manganic complex couple. The potential of a platinum electrode in a similar solution in which one-half of the complex had been reduced with ferrous sulfate solution was about +0.78 volt (S. C. E.) when the pH was 2.06.

Effect of pH

The pH may be of influence for several reasons. From the Nernst equation it is readily seen that the oxidation potential of lead dioxide is a function of the pH. Theoretically the oxidation potential is decreased by approximately 0.12 volt for each unit increase in pH. The kind of acid pyrophosphate which preponderates is determined by the pH, as is the stability of complex ions containing ionizable hydrogen atoms. It will be shown in a subsequent paper that the wave due to the reduction of ferric iron in pyrophosphate solutions is shifted to more negative potentials if the acidity is decreased. This wave interferes if the pH is much smaller than 2.

Experiments 1 to 8, Table I, were performed to determine

TABLE I. EFFECT OF pH

Sample, 5 ml. 0.1 M $MnSO_4$ to produce a final concentration of 5 millimolar in manganese.
Procedure followed, except pH varied. Final pyrophosphate concentration was 0.4 M, agar 0.04 per cent.
Capillary I. $m^{2/3}t^{1/6}$ = 1.314 mg.$^{2/3}$ sec.$^{-1/2}$ at $E_{d.e.}$ = +0.1 volt. Av. i_d/millimolar concn. Mn^{III} = 1.54 microamperes.

Experiment No.	H_2SO_4 (1:1) Used	pH of Final Solution	Color of Final Solution	i_d at +0.1 Volt (S. C. E.) Corrected for Residual Current
	Ml.			*Microamperes*
1	2	....	Brown	7.34
2	4	5.05	Orange brown	7.64
3	4	5.00	Orange brown	7.69
4	4.5	3.78	Violet	7.78
5	5	1.90	Violet	7.82
6	5	1.90	Violet	7.78
7	10	0.90	Violet	7.79
8	20	0.10	Violet	7.84

the pH range in which the violet complex could be quantitatively obtained with a large excess of pyrophosphate present.

Samples containing 5 ml. of 0.1 M manganous sulfate were transferred to a 100-ml. volumetric flask, and 20 ml. of 2 M potassium pyrophosphate were added to each flask to obtain a 0.4 M concentration of pyrophosphate. Various volumes of sulfuric acid (1 to 1) were added to regulate the pH. After addition of 2 ml. of 2 per cent agar and dilution to 100 ml., the oxidation and diffusion current measurements were carried out as in the general procedure.

All the solutions having a pH of 3.78 or smaller had, within experimental error, the same diffusion current and the rich violet color. However, in experiments 2 and 3, the solutions having a pH close to 5 appeared orange red to orange brown in color and the diffusion current was somewhat smaller than at lower pH. After completing the experiments, the pH of the solution used in experiment 4 was varied by adding nitric acid or ammonium hydroxide dropwise. When the pH was increased to 4.52, an orange cast was observed. When the pH was decreased below 4, the complex again appeared violet. A brown precipitate of manganese dioxide always formed in the brown solutions upon standing a few hours. The violet solutions were unchanged after 48 hours.

Effect of Pyrophosphate Concentration

A series of experiments was performed in which the pH was maintained close to 4.0 while the concentration of pyrophosphate was varied. The same final concentration of manganese, 0.005 M, was employed; 0.02 per cent agar was used to suppress the maximum. Before making the polarographic measurements in experiments 1 through 13 in Table II, sufficient potassium nitrate was added to 40 ml. of the oxidized solutions to make the concentration of potassium ions in all solutions 1.6 M. The solutions were then diluted to 50 ml. The oxidation to the trivalent complex was not complete until the pyrophosphate concentration was 0.2 M or 40 times larger than that of manganese.

Experiments 14 through 17 were then performed to determine the maximum concentration of manganese which could be oxidized to the trivalent state, using a final concentration of 0.4 M potassium pyrophosphate. Since a constant large excess of potassium pyrophospate was present, no addition of potassium nitrate or dilution was necessary before making diffusion current measurements. Experiment 14 showed that 10 millimolar manganous ion was quantitatively converted to the trivalent complex at pH 3.9. However, in experiment 15, when the concentration of manganese was increased to 20 millimolar, only 90 per cent of the manganese was converted to the trivalent complex at pH 3.3.

In experiments 16 and 17, 20 millimolar manganous ion and 0.4 M potassium pyrophosphate were again employed but the acidity was increased. In experiment 16, in which the pH was 2.2, a quantitative oxidation to the manganic complex was obtained. In experiment 17, in which a 7 N excess of free sulfuric acid was present, the oxidation to the trivalent complex was also quantitative. However, the complex was not stable in strong sulfuric acid. After 5 days the violet color of the complex prepared in 7 N sulfuric acid had disappeared, while the complex prepared at pH 2.2 showed no appreciable decrease in color.

It may be concluded that manganous ion, up to a concentration of 20 millimolar, can be quantitatively oxidized to the violet manganic complex in a solution 0.4 M in potassium pyrophosphate at a pH of about 2.2. At appreciably lower pH the complex is not very stable and the wave due to the reduction of the ferric complex interferes.

Effect of Air on the Wave

The following experiments were performed to determine the effect of oxygen of the air on the value obtained for the diffusion current.

All the solutions were finally 0.4 M in potassium pyrophosphate. The pH was adjusted to about 2.3 with sulfuric acid (1 to 1), using thymol blue as the indicator. Peptone (0.04 per cent) was used to suppress the maximum. Each solution was treated with 1 gram of lead dioxide.

In experiment 1, Table III, a blank determination was carried out using only the reagents. The measurements were made without removing air from the solution. The measurements were repeated after nitrogen had been passed through the solution rapidly for 2 minutes (columns 2 and 3, Table III). There was no further decrease in the current at these positive potentials after nitrogen had been passed through the solution for a total of 10 minutes.

In experiment 2, the procedure used in the previous experiment was followed but the solution was also 0.004 M in manganous ion before the oxidation. No reduction of oxygen was found at +0.15 volt (S. C. E.). The small value of 0.03 microampere at +0.1 volt (S. C. E.), due to reduction of oxygen, was measured in both the blank and the solution containing manganese. At 0.0 volt (S. C. E.) the effect due to oxygen was no longer the same in the blank and the solution containing manganese. It follows that it is not necessary to remove oxygen if the diffusion current is measured at +0.1 to +0.15 volt (S. C. E.).

These measurements using the manual instrument were substantiated with polarograms obtained with the Heyrovský polarograph. The polarogram (curve 2, Figure 2), was made without oxidizing the manganese and without removing the air. Another portion of the same solution was oxidized with lead dioxide to obtain curve 1. Air was not removed.

TABLE II. EFFECT OF PYROPHOSPHATE CONCENTRATION

Sample, 5 to 20 ml. 0.1 M $MnSO_4$ to produce a final concentration of 5 to 20 millimolar manganese.
Procedure followed except final pyrophosphate concentration varied. Agar, 0.04 per cent, used to suppress maximum. In experiments 1 through 13, 40 ml. of sample diluted to 50 ml. after oxidation and KNO_3 added to obtain 1.6 M potassium ion. Experiments 14 through 17 were not diluted.
Capillary II. $m^{2/3}t^{1/6}$ = 1.967 mg.$^{2/3}$ sec.$^{-1/2}$ at $E_{d.e.}$ = +0.1 volt. Av., i_d/millimolar concn. Mn^{III} = 2.30 microamperes.

Experiment No.	2 M Potassium Pyrophosphate Added	Final Millimolar Pyrophosphate Concn.	Final Millimolar Concn. of Mn	Final pH	Color of Final Solution	True Diffusion Current at +0.1 Volt (S. C. E.) (i_d Appparent − i_r)	Estimation of Mn Oxidized to Trivalent State
	Ml.					*Microamperes*	%
1	0.5	10	5	3.30	Violet brown	3.55	39
2	0.75	15	5	3.30	Violet brown	3.85	42
3	1.00	20	5	3.30	Violet brown	4.10	45
4	1.25	25	5	3.90	Brown violet	4.50	49
5	1.50	30	5	4.09	Brown violet	5.67	62
6	1.75	35	5	4.11	Rose violet	6.37	69
7	2.0	40	5	4.00	Violet	7.19	78
8	3.0	60	5	3.98	Violet	7.50	82
9	4.0	80	5	4.03	Violet	7.85	85
10	5.0	100	5	3.78	Violet	8.35	91
11	7.5	150	5	3.98	Violet	8.82	96
12	10.0	200	5	3.90	Violet	9.18	100
13	20.0	400	5	3.90	Violet	9.17	100
14	20.0	400	10	3.90	Violet	23.0	100
15	20.0	400	20	3.30	Violet	41.4	90
16	20.0	400	20	2.30	Violet	46.2	100
17	20.0	400	20	7 N H_2SO_4	Violet	46.3	100

Table III. Effect of Air on the Wave

Solution 0.004 M Mn, 0.4 M $K_4P_2O_7$, 0.08 per cent peptone, pH = 2.3 (approx.) using H_2SO_4 (1.1) and thymol blue.
Capillary III. $m^{2/3}t^{1/6}$ = 2.150 mg.$^{2/3}$sec.$^{-1/2}$ at $Ed.\ e.$ = +0.1 volt.

	Experiment 1, No Mn Present			Experiment 2, 4 Millimolar Mn^{III}		
E_c (S. C. E.)	Air not removed	N_2 passed through solution 2 minutes	Current due to air in blank	Air not removed	N_2 passed through solution 2 minutes	Current due to air in Mn solution
	Microamperes					
+0.25	−4.35	−4.35	0	4.30	4.30	0
+0.24	−2.86	−2.86	0	5.55	5.55	0
+0.22	−0.56	−0.56	0	....	....	....
+0.20	−0.38	−0.38	0	9.43	9.43	0
+0.15	−0.20	−0.20	0	9.65	9.65	0
+0.1	−0.13	−0.16	+0.03	9.84	9.81	+0.03
+0.075	0.00	....	....	9.89	9.84	+0.05
+0.05	+0.13	−0.10	+0.23	9.98	9.86	+0.12
+0.025	+0.24	....	....	10.06	9.88	+0.18
0.00	+0.41	−0.06	+0.47	10.16	9.90	+0.26

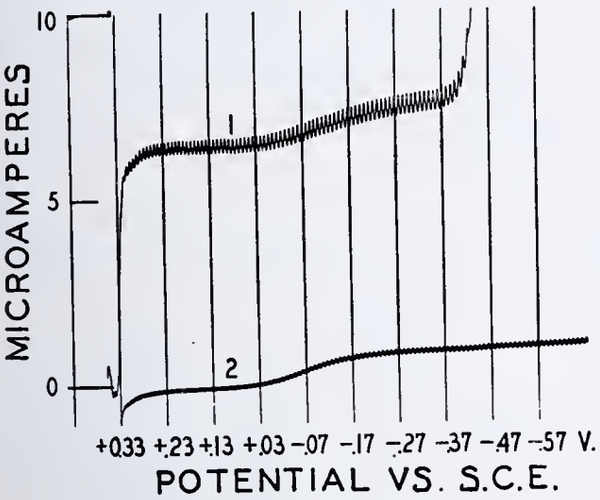


Figure 2. Polarogram

1. 5 millimolar tri-dihydrogenpyrophosphatomanganiate, 0.4 M sodium pyrophosphate, 0.8–0.9 M nitric acid, 0.1 per cent gum arabic, pH = 2.00; oxidized with lead dioxide; air not removed
2. Solution used in 1 before oxidation with lead dioxide

The wave starting at +0.05 volt (S. C. E.) in both curves is due to the first step of the reduction of oxygen. The wave starting at −0.4 volt (S. C. E.) in curve 1 is due to the reduction of the plumbous ion.

Reagents

Potassium Pyrophosphate, 2 M. Slowly pour 132 grams of anhydrous potassium pyrophosphate into about 150 ml. of water while stirring. Dilute to exactly 200 ml.

Nitric Acid (1 to 1). Dilute 100 ml. of freshly boiled concentrated nitric acid with 100 ml. of water.

Sulfuric Acid (1 to 1). Dilute 100 ml. of concentrated sulfuric acid with 100 ml. of water.

Gum Arabic, 2 per cent. Add 1 gram of gum arabic to 49 grams of water and heat to boiling while stirring. This solution is stable for several days.

Peptone, 2 per cent. Dissolve 1 gram of Merck's peptone, "dried", from meat, in 49 grams of boiling water. This solution may be kept in a stoppered bottle containing a few milliliters of carbon tetrachloride, kept cool, or prepared fresh after 48 hours.

Thymol Blue Solution, 0.1 per cent. Mix 0.1 gram of dry thymol blue with 21.5 ml. of 0.01 N sodium hydroxide in a mortar and dilute to 100 ml. with water.

Procedure

Polarographic Determination of Manganese as Tri-dihydrogen Pyrophosphatomanganiate. The sample to be determined may contain from 1 to 100 mg. of manganese as the nitrate, sulfate, or perchlorate in approximately 50 ml. of aqueous solution. Iron may be present in quantities up to 0.2 gram, but chromium, vanadium, and cerium interfere.

Transfer the sample quantitatively to a 100-ml. volumetric flask, and add enough nitric acid (1 to 1) or sulfuric acid (1 to 1) to make the total amount of mineral acid present equivalent to 12 ml. of nitric acid (1 to 1) or 4.5 ml. of sulfuric acid (1 to 1). Slowly add 20 ml. of 2 M potassium pyrophosphate to the solution while swirling. Add 2 or 3 drops of thymol blue. Add more acid or ammonium hydroxide dropwise until the color becomes orange, corresponding to a pH of 2.0 to 2.4. Add 5 ml. of 2 per cent gum arabic or 5 ml. of 2 per cent peptone. Dilute to the mark and mix thoroughly.

Transfer a few milliliters to a polarographic cell and measure the residual current as described below for the oxidized solution. Add about 1 gram of manganese-free lead dioxide for each 25 to 50 mg. of manganese present and shake intermittently for 5 to 10 minutes. Filter through a dry retentive filter paper such as Whatman No. 42 into a clean polarographic cell. Rinse out the cell with the first portion of the filtrate and collect a suitable volume of the filtrate.

Use an external anode. Five milliliters of c. p. carbon tetrachloride may be added to cover the mercury drops collecting at the bottom of the cell. Just before starting the measurements insert the dropping mercury electrode. Measure the current at +0.1 volt and +0.15 volt (S. C. E.) if the manual instrument is used.

If the automatic recording instrument is available, use the 2-volt accumulator. Turn the switch to "anodic and cathodic polarization". The applied potential is zero when the sliding contact is at the exact center of the resistance wire. Regulate the voltage so that each abscissa line corresponds to 0.1 or 0.05 volt. Choose a galvanometer sensitivity such that the wave has a suitable height from +0.1 to +0.15 volt (S. C. E.). Start the polarogram at +0.35 volt (S. C. E.). If iron is present, stop the motor as soon as the light beam moves past the camera, then with the sliding contact disengaged record the zero current line for a distance of 1 or 2 mm. on the sensitized paper. It is desirable to run the residual current on the same polarogram.

Table IV. Proportionality between Diffusion Current and Concentration of Manganese

Capillary I $m^{2/3}t^{1/6}$ = 1.314 mg.$^{2/3}$ sec.$^{-1/2}$ at $Ed.\ e.$ = +0.1 volt.
Procedure used except 0.02 per cent agar used and nitrogen bubbled through solution 2 minutes.

Experiment No.	Final Millimolar Concn. of Complex Manganic Ion	Mn Taken	Apparent Diffusion Current Measured at +0.1 Volt	True Diffusion Current (Corrected for Residual)	Diffusion Current per Millimolar Concentration of Complex
		Mg.	*Microamperes*	*Microamperes*	*Microamperes*
1	0	0	−0.07	....	....
2	0	0	−0.07	....	....
3	0.2	1.1	+0.25	0.32	1.60
4	0.5	2.75	0.69	0.76	1.52
5	1	5.49	1.46	1.53	1.53
6	1	5.49	1.47	1.54	1.54
7	2	10.99	3.02	3.01	1.54_5
8	3	16.48	4.57	4.64	1.54_7
9	5	27.47	7.68	7.75	1.55_0
10	10	54.93	15.40	15.47	1.54_7
11	10	54.93	15.32	15.39	1.53_9
12	20	109.86	30.70	30.77	1.53_9
				Av.	1.54

Proportionality between Diffusion Current and Concentration of Manganese

The results reported in Table IV show that proportionality exists between diffusion current and the concentration over a wide range of concentrations.

The deviation from the average was more than 1 per cent only when very low concentrations of manganese were used.

The determinations were made according to the general procedure. A few polarograms are given in Figure 3. Even at very low manganese concentrations, oxygen from the air does not interfere.

Structure of Mangani Pyrophosphoric Acid

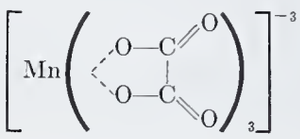
If manganic orthophosphate is dissolved in meta, pyro, or sirupy orthophosphoric acid, violet-colored complex compounds are formed. These compounds have been named mangani metaphosphoric acid, mangani pyrophosphoric acid, and mangani orthophosphoric acid, depending on the acid in which they were prepared.

A study of the literature reveals that the following types of trivalent manganese compounds have been crystallized from solutions of the various acids:

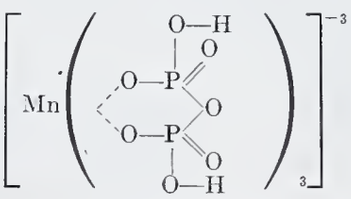
1. From orthophosphoric acid
   $MnPO_4.H_2O$, green gray in color (*4*)
2. From pyrophosphoric acid
   $Mn_4(P_2O_7)_3.14H_2O$, pale violet (*1*)
   $NaMn(P_2O_7).5H_2O$, pale red (*11*)
3. From metaphosphoric acid
   $Mn(PO_3)_3.H_2O$, red (*1*)

Rosenheim (*11*) observed that many compounds of the type M^I, $MnP_2O_7.3H_2O$, crystallized from solutions containing the mangani pyrophosphoric acid. He suggested the formula $\left[Mn\,{}^{P_2O_7}_{(H_2O)_3}\right]^-$ for the anion of the violet complex acid in aqueous solution. This formula has been adopted in the literature. The assumption of similarity between the complex in solution and the crystalline form obtained is not justified.

The violet manganic complex with pyrophosphate described in this paper has properties which are very similar to that of the trioxalatomanganiate complex. The latter has the following structure:



which is stabilized by the five-membered chelate ring. A similar structure with a six-membered chelate ring is obtained when the dihydrogen pyrophosphate ion combines with manganic:



Various observations substantiate the conclusion that the dissolved violet complex dealt with in this paper is the tri-dihydrogen pyrophosphatomanganiate of the above structure.

The pH of an alkali dihydrogen pyrophosphate solution is about 4.25. As is evident from Table I, the violet complex is stable at this and lower pH. In order to obtain a stable solution of the violet complex it is necessary to have present a large excess of dihydrogen pyrophosphate. This indicates that the violet complex has ionic bonding and that it has a relatively small stability constant. Cartledge and Nichols (*3*) in their study of trimalonatomanganiate arrived at a similar conclusion.

When the pH is of the order of 5 or larger dihydrogen pyrophosphate ions in the complex may be replaced by water molecules, producing the brown-colored complex. This behavior has been observed by Cartledge *et al.* (*2*, *3*) in the study of trimalonatomanganiate and the trioxalatomanganiate complexes.

Tomula and Aho (*13*) found in transport measurements that the violet manganic pyrophosphate complex migrates toward the anode. This observation eliminates the possibility that pyrophosphoric acid occupies the coordination

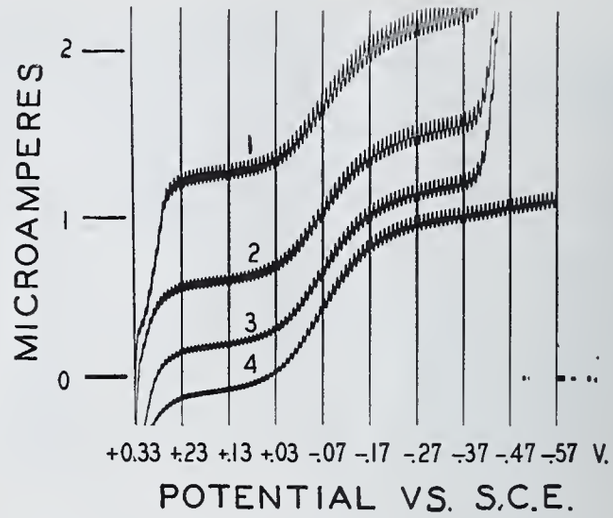


Figure 3. Polarogram

1. 1 millimolar tri-dihydrogenpyrophosphatomanganiate, 0.4 *M* sodium pyrophosphate, 0.8–0.9 *M* nitric acid, 0.1 per cent gum arabic, pH = 2.00; air not removed
2. Like 1 but 0.5 millimolar Mn^{III}
3. Like 1 but 0.2 millimolar Mn^{III}
4. Residual current. Solution used in 3 before oxidation with lead dioxide

Table V. Diffusion Coefficient of Violet Complex

Capillary No.	m *Mg./sec.*	t at +0.1 Volt *vs.* S. C. E. in 0.4 *M* Pyrophosphate at pH 2.3	$m^{2/3}t^{1/6}$ at +0.1 Volt	i_d per Millimole per Liter	$D \times 10^5$ *Sq. cm./sec.*
I	1.051	4.10	1.31	1.54	0.375
II	2.111	2.92	1.97	2.30	0.373
III	2.625	2.08	2.15	2.50	0.369
				Av.	0.373 ± 0.003

places in the complex. The possibility that trihydrogen pyrophosphate ions occupy the coordination positions is not considered probable, because an alkali trihydrogen pyrophosphate solution has a pH of about 1.40 and the complex was found stable at a pH of 4. Evidence that the complex is tri-dihydrogen pyrophosphatomanganiate was obtained from the calculation of the diffusion coefficient of the complex ion. The diffusion coefficient was calculated from the polarographic results with the aid of the Ilkovic equation:

$$i_d = 605\, n\, C\, D^{1/2} m^{2/3} t^{1/6}$$

By titration with ferrous sulfate it had been found that the manganese in the complex is present in the trivalent form. Hence n in the above equation is equal to one, assuming that the complex ion contains one manganic ion.

The diffusion coefficient was calculated from three sets of measurements with three different capillaries. The results are summarized in Table V.

For the sake of comparison the authors have also calculated the diffusion coefficient of the trioxalatocobaltiate ion from its diffusion current using capillary I. The medium was 1 *M* in potassium oxalate, 0.2 *M* in ammonium acetate, and 0.5 *M* in acetic acid, and contained 0.018 per cent gelatin. The solution had a pH of 5.0. The average value of the diffusion coefficient calculated was 0.523×10^{-5} sq. cm. per second.

Using Jander's (*9*) expression for the relation between the molecular weights and diffusion coefficients of two closely related species x and k:

$$M_x = \left(\frac{Z_k D_k}{Z_x D_x}\right)^2 M_k$$

in which Z is the specific viscosity of the solution, D, the diffusion coefficient, and M the molecular weight, it is possible to calculate the molecular weight of the manganic pyrophosphate complex. The viscosities of the media in which the diffusion currents of the manganiate and cobaltiate complexes were determined were nearly identical.

The formula of the oxalatocobaltiate has been proved (*2*) to be $Co(C_2O_4)_3^{---}$, corresponding to a molecular weight of 323. Substituting the various values in the above equation we find:

$$M_x = \frac{(5.23 \times 10^{-6})^2}{(3.75 \times 10^{-6})^2} \times 323 = 628$$

Assigning to the manganic dihydrogen pyrophosphate complex the formula $[Mn(H_2P_2O_7)_n^{3-2n}]$ we calculate from the molecular weight of 628 a value of $n = 3.25$. Since Jander's equation is only approximate we may consider the agreement between the expected value of $n = 3$ and the calculated one of 3.25 as satisfactory. The result of the molecular weight calculation indicates that the complex has the formula $[Mn(H_2P_2O_7)_3]^{---}$.

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A Modified Stock Valve

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THE use of porous disks in combination with a mercury cutoff as a stopcock substitute was first suggested by Stock (*2*), who used two disks. A modified type of valve using only one disk, to permit more rapid passage of gas through the valve, was constructed by Schumb and Crane (*1*).

The valve described uses one sintered-glass disk and has been designed to have a minimum of dead-end space and to withstand a pressure difference of one atmosphere in either direction without use of a barometric height of mercury. It has been used for pure gases only. Passage of a gas mixture with components of widely different molecular weights through the valve might lead to changes in the composition of the mixture. This new valve is more difficult to construct than the design of Schumb and Crane and is not so suitable for controlling the flow of liquids.

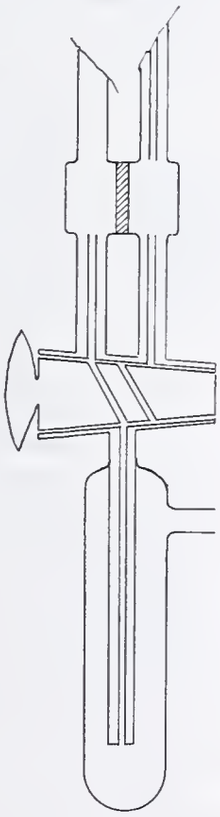

Figure 1

The construction of the valve is obvious from Figure 1. The sintered disk must have pores sufficiently fine so that mercury will not flow through the disk at the pressure differences which usually exist across the valve. If the valve is to withstand a pressure difference of one atmosphere, the commercially available disks with the smallest pores (graded as either D or F) should be used. For pressure differences lower than one atmosphere, coarser disks may be employed advantageously as they offer a lower resistance to the flow of gas through the valve. The sintered disk was obtained already sealed in a glass tube. In making seals near the sintered disk, the tubing and the disk should be heated slowly and uniformly. It is convenient to make the tubes leading to the mercury reservoir so long that when the line is evacuated, all the mercury in the valve can be withdrawn with a pressure of 30 mm. in the reservoir. Then a water aspirator may be used to operate the valve.

To close the valve, mercury is admitted to the compartment which is at the higher pressure. The gas pressure forces the mercury against the sintered disk and prevents flow of gas through the disk. If the pressure on one side of the valve fluctuates during the experiment above and below the pressure on the other side, mercury should be placed in both compartments. When both compartments have been filled with mercury, and the valve is to be opened, mercury should be withdrawn first from the compartment which is at the lower pressure. This procedure avoids rapid bubbling of the gas through the mercury and the possible spraying of liquid mercury through the line.

This valve possesses the advantages and the disadvantages of a mercury cutoff, such as freedom from leaks, no contact between gas and stopcock lubricant, and contamination of the gas with mercury vapor. In comparison with the conventional mercury cutoff, it is more compact and requires less mercury. However, the sintered disk offers considerable resistance to gas flow. To measure the magnitude of this effect, a series of flow tests was made on air using a 20-mm. Pyrex disk, No. 39570 F. The volume of air flowing through the disk in definite time intervals was measured with a wet gas meter, and the pressure difference across the disk was obtained with a dibutylphthalate manometer. The pressure difference across the disk was varied over the range 3.4 to 0.5 mm. of mercury. The experimental rate of flow was approximately constant over the given pressure range and was 6.5 cc. of air (25°, 1 atmosphere) per minute per mm. of pressure difference. As the measurements were made at a pressure near 1 atmosphere, it may be assumed that the pore diameter is large, relative to the mean free path of the gas molecules and that the flow is isothermal. Calculations show that a disk 2 mm. thick is equivalent to a capillary tube 2 mm. long and 0.16 mm. in diameter. Obviously, evacuation will take longer with this valve in the vacuum line than with the usual stopcock. The speed of evacuation may be increased by using larger disks. However, for kinetics investigations, traces of oxygen or other unwanted gases can be removed by flushing the reaction cell five or six times with the reactant gas. The flushing procedure is just as effective and takes much less time than prolonged evacuation

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Lipoid Oxidase Studies

A Method for the Determination of Lipoxidase Activity

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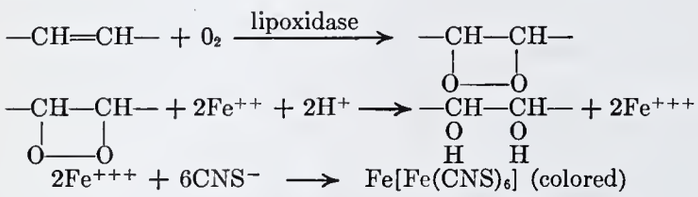
THE work of Sumner and Sumner (*4*) proved that the bleaching of carotene by the enzyme "carotene oxidase" is an indirect result of the action on unsaturated fats of a fat-peroxidizing enzyme. The existence of this lipoxidase had been noted by a few workers (*1, 2, 3*).

A method for the quantitative determination of enzymic peroxidation has been devised. It was found that the fat peroxides in dilute water suspension oxidize ferrous iron, and the ferric iron formed can be determined colorimetrically as the thiocyanate, according to the following reactions (*5*):



Details of Method

In a clean, dry 250-cc. Erlenmeyer flask are placed 5 mg. of linoleic acid dissolved in 5 cc. of acetone, and 100 cc. of water and 5 cc. of citrate buffer (pH 6.5) are added. [The buffer was made by mixing 55 cc. of a solution of citric acid (21.008 grams per liter) and sodium hydroxide (8 grams per liter) with 45 cc. of 0.1 *N* sodium hydroxide.] This mixture is brought to 25° C., and 1 cc. of aqueous enzyme extract is added and allowed to react for the desired time, the flask being rotated to produce adequate agitation. Ten cubic centimeters of concentrated hydrochloric acid are added to stop the reaction and to provide an acid medium for the oxidation of the ferrous iron by the fat peroxide, and 1 cc. of a 5 per cent solution of ferrous ammonium sulfate in 3 per cent hydrochloric acid is added. Either of the procedures discussed below may then be used.

Table I. Determination of Lipoxidase Content

Enzyme Source	Galvanometer Reading	Fe^{+++} $\gamma/cc.$	Net Fe^{+++} $\gamma/cc.$	Total Fe^{+++} γ	Time of Reaction *Min.*	Weight of Enzyme Source *Gram*	Lipoxidase Content *Units/gram*
1. Soybean meal							
No. 1	19.6	2.99	2.37	607	0.5	0.01	34,700
Blank	64.0	0.62				0.01	
No. 2	19.3	3.03	2.41	617	0.5	0.01	35,200
Blank	64.0	0.62				0.01	
2. Green beans							
No. 1(a)	14.4	3.67	2.91	746	7	0.5	72.0
No. 1(b)	28.3	2.20	1.44	369	4	0.5	
Blank	59.3	0.76				0.5	

A. Determination of Ferric Thiocyanate in Alcohol-Water Solution. Ten cubic centimeters of the above mixture are transferred to the colorimeter cell. Exactly 15 minutes after the addition of the ferrous salt (15 minutes was found necessary for complete decomposition of the fat peroxide), 10 cc. of ethyl alcohol are added to the contents of the cell. This removes the turbidity produced by the presence of the fat. One cubic centimeter of 20 per cent ammonium thiocyanate is added and the colorimeter cell (a large test tube) is shaken. The tube is placed in the colorimeter and the galvanometer scale reading is recorded. [Wratten filter No. 65 (green) was used in the colorimeter.] Atmospheric oxidation of the ferrous iron produces a slow drift in the scale reading if the solution stands for any length of time.

B. Determination of Ferric Thiocyanate in Isoamyl Alcohol. Twenty cubic centimeters of the oxidized fatty acid solution are transferred by means of a pipet to a 100-cc. test tube, and exactly 5 cc. of isoamyl alcohol are added. Then 5 cc. of a solution of 20 per cent ammonium thiocyanate are added, and the test tube is stoppered and tipped slowly upside down about six times. This gives complete extraction of the color without producing emulsions or turbidity. The layers are allowed to separate, 5 cc. of isoamyl alcohol are added and uniformly mixed by gentle swirling of the tube, and 5 cc. of the red isoamyl alcohol layer are pipetted into a 25-cc. volumetric flask. The flask is filled to the mark with isoamyl alcohol. The color may be measured in a photoelectric colorimeter, as in procedure A, or in a Duboscq colorimeter.

In either procedure the ferric iron content of the solution is determined in micrograms per cubic centimeter by reference to a standard curve, or by comparison with the color formed by a standard iron solution. From these data the total ferric iron in the reaction mixture may be calculated. A simple straight-line graph is convenient for this calculation.

With either method, a blank determination must be used to compensate for the iron in the reagents and for atmospheric oxidation during the manipulation. The blank determination is carried out exactly as above, except that the enzyme extract is added after the solution has been acidified, so that there is no enzyme action. The ferric iron value obtained is subtracted from the value for the active reaction mixture.

Procedure A is rapid and economical, but requires the use of a photoelectric colorimeter, since the red color in water and ethyl alcohol is not sufficiently stable for use in a visual colorimeter. Procedure B is designed for application of the method when a photoelectric instrument is not available.

Application of the Method

On the basis of this analytical technique, a unit of lipoxidase was defined as the activity which, in the presence of 5 mg. of linoleic acid in the described mixture, catalyzes the reaction of 1 microgram of oxygen in 1 minute at 25° C. at pH 7.

Using aqueous suspensions of the pulverized materials as enzyme extracts, the lipoxidase content of various enzyme sources was compared. Since in the early stages the amount of peroxidation was not directly proportional to the time of reaction, it was necessary to establish the configuration of the reaction curve in each case. The enzyme concentration was calculated from a time interval lying on the straight-line portion of the curve. A collection of illustrative data is presented in Table I.

In Table I, the galvanometer readings indicate the amount of light transmitted by the ferric thiocyanate solution. The calibration curve formed by plotting the galvanometer readings against the ferric thiocyanate concentration is logarithmic in form. Hence, at high color concentrations the sensitivity of the method decreases, the useful limit of concentration being about 4 micrograms of ferric iron per cc.

It is obvious from Table I, that the use of a short reaction time and a diluted enzyme extract multiplies the error when calculating the lipoxidase concentration, although in such

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Table II. Quantitative Comparison of Lipoxidase Sources

Enzyme Source	Lipoxidase Unitage per Gram Fresh weight	Dry weight
Soybean meal[a]	..	35,000
Peas (green Telephone)	1,400	6,220
Potatoes (fresh Irish Cobbler)	378	2,150
Wheat germ	..	810
Green string beans	71	790
Alfalfa, freshly cut	26	90
Asparagus (Green Jumbo)	7.2	74

[a] Dry Seneca soybeans, ground and thoroughly extracted with petroleum ether at 25° C.

cases the reaction curve can be considered to be a straight line, which simplifies the determination. In the second example presented, the enzyme concentration was calculated from a 3-minute interval lying on the straight-line portion of the curve formed by plotting peroxidation against time.

Table II presents the results of the investigation.

Summary and Conclusions

A rapid colorimetric method for the determination of the activity of the enzyme lipoxidase is based upon the fact that the resulting fat peroxide oxidizes ferrous iron. The ferric ion formed is determined as the colored thiocyanate complex by means of a photoelectric colorimeter.

The lipoxidase content of several vegetables was compared by means of this method.

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Separation and Determination of Protein Sulfur, Sulfide Sulfur, and Other Sulfur

In Sodium Sulfide Dispersions of Keratins

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During a study by the junior author of the dispersion of certain keratins in solutions of sodium sulfide, it became desirable to determine separately the protein sulfur, sulfide sulfur, and, as a third fraction, other sulfur compounds. A review of the literature failed to provide a method suitable for such a separation. Theis and Ricker (*9*) studied the inorganic sulfur compounds produced during prolonged action of alkaline sulfide solutions on cowhide, but they reported no work on the protein sulfur.

Several methods for obtaining the desired separation were tried, including treatment of the dispersion with mineral acids, with lead acetate, and with trichloroacetic acid.

Treatment of the dispersion with mineral acids would release hydrogen sulfide from sulfides present, but this treatment would also release sulfur dioxide from sulfites and bisulfites and, if the acid were sufficiently concentrated, sulfur from thiosulfates. The acid concentration required to precipitate the protein was sufficiently high to release sulfur dioxide from sulfites and precipitate sulfur from thiosulfates.

Precipitation of the dispersion with normal lead acetate, filtering, and washing with hot ammonium acetate and then with hot water separated the other sulfur compounds from the protein and lead sulfide formed but provided no means of separating the two latter groups.

The protein dispersion is colloidal, and mineral salts are effective in flocculating the disperse phase. As aluminum salts are very effective in flocculation (*6*) a water suspension of basic aluminum acetate was found to precipitate the protein present and also to liberate hydrogen sulfide from the sodium sulfide present. The use of basic aluminum acetate was also found to be applicable to the separation of sulfides from sulfites, thiosulfates, and sulfates.

Three dispersions were analyzed by the method discussed in this paper.

The protein materials used were chicken feathers, hog hair, and wool. The sulfur contents of these materials, based upon their air-dry weight, were: chicken feathers, 2.3 per cent; hog hair, 5.6 per cent; and wool, 3.6 per cent. The keratin dispersions were prepared by a modification of the method described by Goddard and Michaelis (*4*). These dispersions contained about 7 per cent of the protein material in 0.1 *M* sodium sulfide. Total sulfur was determined in the protein materials by an alkaline permanganate fusion (*8*). The official magnesium nitrate ignition (*2*) and the alkaline permanganate fusion (*8*) were used on the precipitated proteins, and the two methods gave the same results. Magnesium nitrate did not wet the chicken feathers, and so was not used for the determination of total sulfur in this material.

The basic aluminum acetate method was also tried on solutions of sodium sulfide, both by itself and mixed with some other sulfur salts, to determine whether it would be successful in separating sulfide sulfur from other sulfur compounds. Also, sodium sulfide and other sulfur salts were added to the feather dispersion and the sulfide sulfur was separated by the aluminum acetate.

Reagents

Basic aluminum acetate, suspension of 5 grams in 100 ml. of water.

Lead acetate, basic, dry powder, a 1 per cent solution, as for sugar analysis by the Horne method.

Bromine–hydrobromic acid, equal volumes of 48 per cent hydrobromic acid and bromine.

Magnesium nitrate, dissolve 150 grams of magnesium oxide in nitric acid (1 + 1), avoiding an excess of acid. Add a little magnesium oxide in excess, filter from excess of magnesium oxide, iron oxide, etc., and dilute to 1 liter (*1*).

Potassium permanganate, a saturated solution.

Sodium hydroxide, a 15 per cent solution; also solid.

Nitrogen, compressed.

Hydrochloric acid, concentrated and diluted 1 to 1 with water.

Sodium carbonate, solid, anhydrous.

Methyl orange, water solution, 0.5 gram per liter.

Barium chloride, a 10 per cent solution.

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Carbon disulfide, analytical reagent.

N-Octadecyl alcohol, a saturated solution in 95 per cent ethyl alcohol.

Ammonium hydroxide, concentrated.

Procedure

A 2-ml. sample (weighed) of the keratin dispersion was washed into a flat-bottomed vial (*D*, Figure 1) with the minimum amount of water necessary and 5 ml. of *n*-octadecyl alcohol reagent were added to minimize foaming. Basic aluminum acetate suspension was added, the mixture was heated, and the hydrogen sulfide evolved was swept with nitrogen into a basic lead acetate solution. This reaction was carried out in the apparatus shown in Figure 1.

Two milliliters of the basic aluminum acetate were drawn into the pointed tube, *C*, a clamp at *A* was tightened and another at *B* loosened, the T-tube was connected, and *C* was lowered into the solution in vial *D*. Nitrogen was then turned on so that 3 or 4 bubbles a second passed through the water in bottle *H*. Connections were completed with test tubes *F* and *G*; vial *D* was lowered into boiling water in the 400-ml. Griffin beaker, *E*; the clamp at *A* was loosened and that at *B* tightened, whereupon the stream of nitrogen drove the basic aluminum acetate into the dispersion, precipitating the protein and evolving hydrogen sulfide from sulfides present. The hydrogen sulfide was carried into the basic lead acetate solution in *F*, precipitating lead sulfide. Tube *G* also contained basic lead acetate and was used only as a precaution. All the hydrogen sulfide was driven off in 5 to 10 minutes and further heating was avoided, since it caused the protein to stick to vial *D*. The stopper containing tube *C* was removed from *D* while the nitrogen was still bubbling through it to prevent protein material from collecting inside the tube.

The protein was broken up with a rubber-tipped stirring rod, transferred to filter paper in a Büchner funnel, and washed thoroughly with hot water. The final volume of the filtrate was usually about 250 to 300 ml. The protein was then ignited with about 15 ml. of magnesium nitrate solution or with 10 ml. of permanganate solution plus 1 ml. of 15 per cent sodium hydroxide.

The alkaline permanganate fusion was made at 600° C. instead of at 500° C. as in the original method (*8*), but after fusion the mixture was treated as directed by Pollock and Partansky (*8*). The magnesium nitrate ignition mixture was analyzed by the A. O. A. C. method (*2*). After the ignition mixture was dissolved, the solution was made just acid to methyl orange by the proper use of ammonium hydroxide and hydrochloric acid. About 0.5 ml. of hydrochloric acid per 100 ml. of solution was then added and the solution was heated to boiling. Two milliliters of the barium chloride solution were then slowly added from a pipet, with constant stirring of the solution. The solutions were kept at about 40° C. overnight, filtered through weighed Gooch crucibles, and the precipitated barium sulfate was washed with hot water. The barium sulfate was then ignited at a cherry red heat in a muffle furnace (about 650° C.) to constant weight, cooled for an hour in a desiccator, and weighed.

The filtrate from the precipitated protein contained the other sulfur compounds. To it were added about 5 grams of sodium hydroxide and 5 ml. of bromine water. The solution was kept alkaline (*3*). It was heated on a steam bath for an hour, acidified with hydrochloric acid (1 + 1), and boiled to expel bromine, and the sulfur was determined as before.

The sulfide sulfur was determined by dissolving the lead sulfide in tube *F* (Figure 1) in 3 ml. of bromine–hydrobromic acid mixture, heating gently until all bromine was driven off, then cooling and neutralizing with sodium carbonate. Tall-form beakers were used to avoid loss by effervescence. An excess of about 1 gram of sodium carbonate was added and the solution was boiled for about 15 minutes. Lead carbonate was precipitated and sodium sulfate remained in solution. The lead carbonate was filtered off and freed from sodium sulfate by washing. The filtrate was then made slightly acid with hydrochloric acid (1 + 1) and the sulfur was determined as before.

The amount of basic aluminum acetate necessary to precipitate the protein and drive off the hydrogen sulfide was determined by treating the dispersion with measured quantities of basic aluminum acetate, boiling until the hydrogen sulfide was all evolved, and testing the solution with a drop of 10 per cent normal acetate. The time necessary to remove the hydrogen sulfide was also determined by testing at intervals with lead acetate paper. Care was taken to have all rubber and glass connections perfectly dry before the operation was started, so that no hydrogen sulfide would be dissolved in the water present.

Preliminary determinations of the sulfur content of methionine and cystine were made by the official magnesium nitrate method and the alkaline permanganate fusion method. The results, given in Table I, show that a fusion temperature of 600° C. must be used with the alkaline permanganate fusion method to obtain the true amount of sulfur in methionine. The results obtained by this method compared favorably with those obtained by the magnesium nitrate ignition.

Results and Discussion

Three sodium sulfide dispersions of keratins were analyzed for total sulfur by fusion and the three fractions of sulfur compounds separated from each dispersion by the use of basic aluminum acetate were also analyzed for sulfur. The analyses were made in duplicate or triplicate on freshly prepared dispersions. Analyses were also made of dispersions

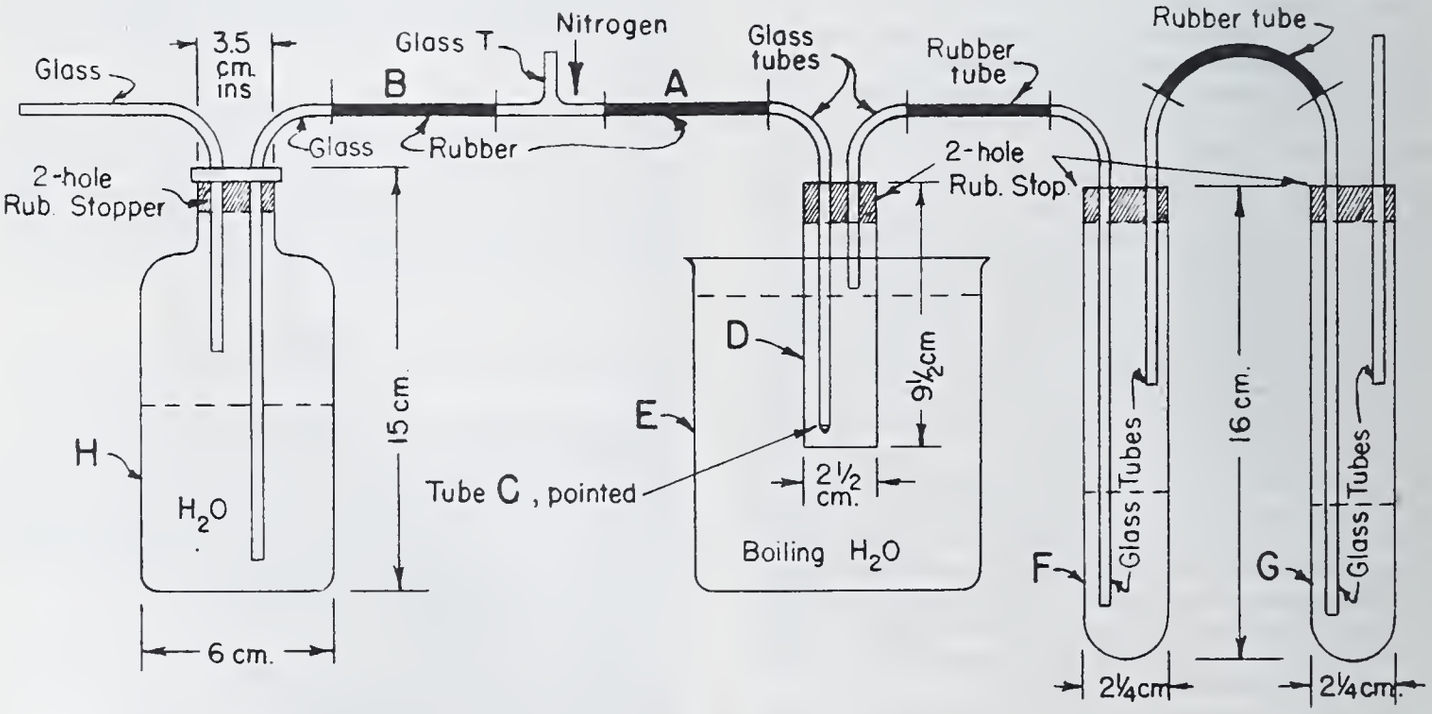

Figure 1. Diagram of Apparatus

Table I. Determination of Sulfur

(Comparison of magnesium nitrate method and alkaline permanganate method)

Material	Sulfur Obtained: Magnesium nitrate method	Alkaline permanganate method 500° C.	Alkaline permanganate method 600° C.	Sulfur theoretical
	%	%	%	%
d,l-Methionine	21.52	16.97	21.56	21.47
	21.55	16.98	21.53	...
l-Cystine	26.20	...	26.40	26.63
	26.36	...	26.44	...

Table II. Basic Aluminum Acetate Separation of Sulfur in Sodium Sulfide Dispersions of Keratins

	Protein Sulfur	Sulfide Sulfur	Other Sulfur	Sum	Total Sulfur by Fusion Method
	Mg./g.	*Mg./g.*	*Mg./g.*	*Mg./g.*	*Mg./g.*
Feather dispersion, freshly prepared	2.3	2.1	1.1	5.5	5.3
	2.4	1.9	1.1	5.4	5.5
Feather dispersion, stood 1 month	1.8	None	3.6	5.4	...
Hog hair dispersion, freshly prepared	4.1	1.5	0.7	6.3	6.1
	4.4	1.3	0.7	6.4	6.1
	4.1	1.4	0.6	6.1	...
Hog hair dispersion, stood 15 days	4.2	None	2.2	6.4	...
	4.1	None	2.2	6.3	...
Wool dispersion, freshly prepared	3.2	1.5	0.6	5.3	5.6
	3.4	1.7	0.5	5.6	5.5

that had stood in the laboratory for some time. The results of these analyses are given in Table II.

To test for the possible formation of elemental sulfur in the protein fraction upon standing, the protein was separated from duplicate samples of a hog hair dispersion that had stood in the laboratory for 15 days. One protein fraction was analyzed as usual; the other was washed twice with boiling carbon disulfide, after which it was dried and analyzed for sulfur. The results were nearly identical, showing that no sulfur had been removed from the second protein fraction; moreover, the residue from the carbon disulfide used in washing contained no sulfur.

The pH of a freshly prepared feather dispersion was 12.0. One milliliter of this material was diluted with 5 ml. of water (resulting pH 10.6) and boiled with 2 ml. of basic aluminum acetate (pH 4.5) until all the hydrogen sulfide was driven off. The solution was then cooled and again tested for pH, which was found to be 4.5.

Mixtures of the feather dispersion and various sulfur compounds were analyzed by the basic aluminum acetate method as described. Recovery of the added sulfur was complete and the amounts of protein sulfur and sulfide sulfur in the dispersion were not affected. When sodium sulfide was added to the dispersion, the protein sulfur remained unchanged, the sulfide sulfur increased by the amount present in the sodium sulfide, and the other sulfur fraction also increased by the amount present in the added salt. When solutions of sodium sulfite, sodium thiosulfate, and potassium sulfate were added to the protein dispersion, analyses showed that the protein and sulfide sulfur of the dispersion remained unchanged, the sulfur of the added salt being included in the remaining sulfur of the dispersion. Sodium bisulfite, when added to the dispersion, did not behave like the other added salts; an increase was observed in both the protein and sulfide sulfur fractions. Results of the analyses of mixtures of the protein dispersions with added sulfur compounds are given in Table III.

The sulfur distribution of a keratin dispersion in sodium sulfide solution is probably the result of an equilibrium between several reactions involving the inorganic sulfide ion and the disulfide sulfur of the protein. Part of the original sulfide is oxidized to disulfide or to a polysulfide during reduction of the cystine residues of the keratin (*4*). Another reaction that would also result in a decrease of the sulfide-ion concentration is that described by Nicolet and Shinn (*7*). In this reaction a part of the original sulfide sulfur is incorporated into the protein molecule by addition to the double bonds formed by the action of alkali on the hydroxy amino acid residues of the original protein. On the other hand, it is possible that the final sulfide sulfur might include that part of the cystine sulfur which appears as inorganic sulfide during the conversion of cystine to lanthionine—a conversion which, as Horn and Jones (*5*) have shown, occurs upon treatment of proteins with sodium sulfide.

It is evident from the foregoing discussion that no conclusion can be drawn from the data in Table II as to the sources of the sulfur in the three fractions. Indeed, this problem lies outside the scope of the present paper. However, it is hoped that the method of separation and determination of the sulfur fractions described here may be a useful tool (with cystine analyses, for example) in studies of the reactions involved in the dispersion of keratins by sodium sulfide and of the action of alkali on the sulfur of proteins.

Table III. Basic Aluminum Acetate Separation of Sulfur in Keratin Dispersions Plus Added Sulfur Compounds

	Protein Sulfur	Sulfide Sulfur	Other Sulfur	Sum	Total Sulfur[a]
	Mg.	*Mg.*	*Mg.*	*Mg.*	*Mg.*
Protein dispersion (1 gram)	2.3	2.1	1.1	5.5	5.4
	2.4	1.9	1.1	5.4	5.5
Sodium sulfide (1 ml.)	None	2.2	1.4	3.6	3.4
	None	2.3	1.4	3.7	3.6
Protein dispersion (1 gram) + sodium sulfide (1 ml.)	2.3	4.2	2.4	8.9	9.4
	2.4	4.1	2.5	9.0	9.2
Protein dispersion (1 gram) + sodium thiosulfate (1 ml.)	2.5	1.9	7.7	12.1	11.9
	2.3	2.0	7.9	12.2	12.2
Protein dispersion (1 gram) + sodium sulfite (1 ml.)	2.4	1.9	2.7	7.0	6.9
	2.3	2.0	2.6	6.9	6.9
Protein dispersion (1 gram) + potassium sulfate (1 ml.)	2.5	2.0	4.4	8.9	8.6
	2.4	1.9	4.5	8.8	8.7
Protein dispersion (1 gram) + sodium bisulfite (1 ml.)	2.8	2.9	1.2	6.9	6.7
	2.9	2.6	1.1	6.6	7.0

[a] Alkaline permanganate fusion method.

Summary of Results

The sulfur in sodium sulfide dispersions of keratins may be separated into three parts: protein sulfur, sulfide sulfur, and other sulfur compounds by means of basic aluminum acetate.

Sulfides may be separated from sulfites, thiosulfates, and sulfates, but not from bisulfites, by treatment with basic aluminum acetate.

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Determination of Pure Carotene in Plant Tissue

A Rapid Chromatographic Method

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A RESEARCH project at this laboratory involved the accurate determination of the carotene content of many samples of fresh and dehydrated vegetable products. (Carotene referred to in this paper includes both alpha- and beta-carotene, in most cases approximately 10 per cent alpha and 90 per cent beta.) The methods commonly used at present for the determination of carotene in plant materials depend upon the phasic separation of carotene from interfering pigments. The carotene is extracted from the plant material with solvents, such as alcohol or acetone, and then usually removed from the original extractant with petroleum ether. A number of workers (*1, 3, 4, 9*) have shown, however, that the petroleum ether phase obtained from dehydrated and stored plant materials or silage may still contain, after final purification, noncarotene chromogens, which are estimated as carotene.

Several methods (*1, 2, 4*) have been published in which the noncarotene chromogens are removed by contact adsorption or by passage through a Tswett column. In these procedures it is often necessary to activate the adsorbent and to carry out preliminary phasic separation with petroleum ether from the original solvent.

A procedure has been devised at this laboratory for the determination of carotene in dehydrated plant products with an adsorbent that requires no special activation and no phasic separation. The steps in brief consist of (1) extraction with a mixture of 30 per cent acetone and 70 per cent Skellysolve B, (2) removal of most of the acetone by evaporation, (3) separation of the carotene from interfering pigments by passage through an adsorption column of activated magnesium oxide, and (4) washing with 3 to 5 per cent acetone in Skellysolve B. The adsorbent is a modified mixture of Micron Brand activated magnesium oxide No. 2641 and Hyflo Super-Cel, first introduced by Strain (*8*) for the chromatographic separation of various carotenoid pigments.

Procedure

EXTRACTION OF CAROTENE. Dehydrated plant material (1.00 gram), ground to 40-mesh or smaller, with a carotene range of 50 to 500 micrograms per gram is used for the determination. One-half or 2-gram samples should be taken if the carotene content deviates from this range. The apparatus used at this laboratory is either a 300-ml. standard taper Erlenmeyer flask with a condenser and heated on a hot plate, or a Soxhlet apparatus with a 500-ml. Soxhlet flask. The material is refluxed for half an hour with 100 ml. of a mixture of 30 per cent acetone and 70 per cent Skellysolve B in the first apparatus, or for 1 hour with 200 ml. if the Soxhlet apparatus is used, operating at a rapid siphon rate. If the former apparatus is used, the sample is filtered on a sintered-glass funnel (coarse porosity) into a 500-ml. filter flask and washed with approximately 25 ml. of the Skellysolve three or four times. The filter flask, or the Soxhlet flask, containing the extract is placed directly in a steam bath and evaporated to 25 to 50 ml. Under no circumstance should all the solvent be evaporated, since this would destroy some of the carotene. This extraction procedure removes all the carotene but not all the chlorophyll.

SEPARATION OF CAROTENE FROM OTHER PIGMENTS BY CHROMATOGRAPHIC ADSORPTION. (This laboratory is at present using similar principles in large-scale preparation of carotene.) The adsorbent used is composed of three parts of Hyflo Super-Cel and one part of Micron Brand magnesium oxide, a specially activated product readily obtained on the commercial market at low cost. A 453-gram (1-pound) batch of adsorbent is conveniently prepared by spreading the Super-Cel and magnesium oxide on a large sheet of paper and mixing by turning over the heap approximately ten times, or rotating in a ball mill with the balls omitted.

The adsorption tube is made from a test tube approximately 23 × 200 mm. with a piece of glass tubing attached to the bottom. A plug of cotton or glass wool is put in the bottom, and the adsorption tube is connected to a suction flask or a large vacuum desiccator or suction bell jar. If the two latter devices are used, the receiver may be a 100-ml. or 250-ml. volumetric flask, thus avoiding later transference of the solution to the volumetric flask. With the full vacuum of a water pump, the adsorbent is added until the height of the column is about two-thirds to three-fourths the height of the adsorption tube. All the adsorbent is firmly pressed with a plunger consisting of a glass rod and properly fitting cork, and the column is washed with approximately 50-ml. of Skellysolve, which is then discarded. The evaporated extract is poured on the wet adsorbent column, which removes all the pigments. The flask and column are washed with a mixture of 3 to 5 per cent acetone in Skellysolve B until the solvent comes through colorless. The washings may be continuous or intermittent. Usually a total of 100 ml. is necessary. Full suction must be used with all the operations.

The chlorophyll and xanthophyll are held firmly at the top of the column, as are most of the other noncarotene pigments. The carotene is washed through the column by the acetone-Skellysolve mixture. The acetone is necessary because pure Skellysolve elutes the carotene from the column too slowly to be of value in routine analysis. Occasionally a small amount of noncarotene chromogen may be slowly washed down the column. The carotene is eluted much more rapidly, however, and no interference results, particularly if the final washings are pure Skellysolve B. In most cases the adsorbent may be used a number of times before it is discarded. The whole procedure of adsorption and elution, usually run in duplicate, takes from 5 to 10 minutes.

DETERMINATION OF CAROTENE. The carotene solution is made to volume and determined in any of the usual ways. In this laboratory a Lumetron photoelectric colorimeter provided with an H-4 mercury arc light and with two Corning No. 511 filters plus a neutral gray filter or two Corning No. 511 filters plus a Corning Noviol N 038 filter were used. Since the carotene solution being estimated was relatively pure, both filter combinations gave the same results, but the latter filter set, transmitting at 440 mμ, is intrinsically more accurate. A standard calibration curve was made from S. M. A. carotene that was 90 per cent beta and 10 per cent alpha, purified according to the method of Fraps and Kemmerer (*1*). All the carotene in the samples was estimated as beta-carotene, although a small but variable fraction was alpha-carotene.

Extraction of Fresh Samples

A procedure developed by Moore and Ely (*5*) in which the Waring Blendor is used to extract carotene from fresh plant samples by means of a foaming mixture of alcohol and petroleum ether was tested, and with some slight modifications proved to be excellent.

A 10-gram sample of finely minced plant material is extracted with 150 ml. of 95 per cent ethyl alcohol and 75 ml. of Skellysolve B in a Waring Blendor for 5 to 10 minutes. More alcohol is added if the mixture does not foam. The authors found it convenient to regulate the speed of the blender with a voltage controller. The extract and finely divided plant material are transferred to a fritted-glass filter by means of a piece of wide-bore glass tubing connected to the filter with a rubber stopper. The filter is attached to a suction flask. The container of the blender is alternately washed with alcohol and Skellysolve until the filtrate running into the suction flask is colorless. It was found advisable to remove the alcohol and extracted water from the Skellysolve in a separatory funnel. One hundred milliliters of water containing about 5 grams of sodium sulfate are added to the alcohol-Skellysolve mixture, and the lower aqueous alcohol solution is drawn off. The aqueous solution is extracted three times with 30 ml. of Skellysolve. The sodium sulfate aids in clearing troublesome emulsions, which may occur from time to

Table I. Recovery of Pure Carotene after Passage through Magnesium Oxide Adsorbent

Extinction		
Stock solution	Stock solution adsorbed	Recovered %
0.500	0.510[a]	102.0
0.534	0.532	99.6
0.260	0.260	100.0
0.260	0.255	98.1
		Av. 99.9

[a] Stock solution concentrated in vacuo prior to adsorption.

time. Fifty milliliters of water are poured through the combined petroleum ether fractions, the water is drawn off, and the petroleum ether is concentrated to approximately 25 ml. The magnesium oxide is stable to small amounts of alcohol, so that the prolonged extraction of the petroleum ether with water to remove alcohol, as described by Moore (*4*), is unnecessary. A few grams of anhydrous sodium sulfate are added to the Skellysolve to remove moisture, and a small layer of sulfate is placed above the adsorbent as a precautionary measure. The rest of the procedure is the same as that described for dehydrated material.

Discussion and Results

The various steps in the procedures were carefully checked.

Carotene Recovery. A purified sample of 90 per cent beta and 10 per cent alpha-carotene was used for carotene recovery tests. An aliquot of a stock solution was made to a 100-ml. volume and read in the colorimeter. A similar aliquot was washed through the adsorption column with 3 to 5 per cent acetone in Skellysolve B, made to volume, and read. In other cases the aliquot was made to 100 ml., concentrated in vacuo, and washed through the adsorbent.

The results are shown in Table I.

The recovery of carotene added to samples of dehydrated, stored pea and lima bean vines that had been shown to contain considerable noncarotene chromogens is shown in Table II. These results show that by the extraction and adsorption procedure outlined quantitative recovery of carotene may be obtained both with pure carotene (Table I) and with carotene added to plant samples (Table II).

Effect of Evaporation on Carotene. Data on the effect of vacuum versus steam concentration of the petroleum ether extracts are given in Table III. Carotene is not affected by evaporation on a steam bath under the experimental conditions.

Comparison of the Adsorption Method with Phasic Procedures. The determination of carotene in dehydrated and fresh or frozen plant materials by the adsorption method was compared with the widely used Peterson-Hughes-Freeman (*7*) technique as modified by Peterson (*6*). After the carotene was determined in the Skellysolve extract obtained by the Peterson-Hughes-Freeman method, an aliquot of the same solution was passed through the magnesium oxide absorbent column. Other samples were analyzed directly by the method described here. In this way a comparison of the extraction and determination of carotene by the new technique could be obtained, since the Peterson-Hughes-Freeman technique and its modifications give excellent extraction of carotene. The results shown in Table IV were obtained on samples dehydrated and stored for different periods of time, on others dehydrated and analyzed immediately, and on fresh and frozen samples.

The results in Table IV are typical of those obtained on a large number of samples. It is apparent that the phasic method records as carotene a considerable amount of noncarotene material, since on passing the extracts through the magnesium oxide column lower values are always obtained.

Table II. Recovery of Carotene Added to Dehydrated Plant Samples

Carotene in Sample	Carotene Added	Carotene Theoretical	Carotene Found	Recovered
γ	γ	γ	γ	%
72.6	114.3	186.9	190.0	101.5
8.6	114.3	122.9	122.8	100.0
37.2	114.3	151.5	152.2	100.5
62.8	114.3	177.1	171.5	96.8
77.9	114.3	192.2	190.0	98.8
				Av. 99.5

Table III. Effect of Evaporation on Carotene

Dehydrated Sample	Carotene Vacuum	Carotene Steam
	Micrograms per gram	
Savoy cabbage leaves	280.0	282.0
Broccoli leaves	281.0	270.0
Carrots (vacuum-dried)	486.0	486.0
Carrots (air-dried)	70.4	71.4

Table IV. Carotene Content of Dehydrated and Fresh Vegetable Products

(Determined by the Peterson-Hughes-Freeman technique and by the adsorption procedure)

	Carotene Found		
	PHF procedure	PHF procedure followed by adsorption	Adsorption procedure
	Micrograms per gram		
Dehydrated samples			
Pea vines	73.6	47.0	46.2
Broccoli leaves and petiole	259.0	186.0	192.0
Hubbard squash rind	68.7	39.6	40.0
Pepper	45.7	20.4	20.4
Lima bean leaves	209.0	164.0	170.0
Lima bean vines, laboratory dried	76.0	61.3	59.3
Lima bean vines, commercially dried	53.2	27.8	26.0
Asparagus tops	379.0	334.0	310.0
Spinach	878.0	828.0	810.0
Fresh or frozen samples			
Lima bean silage	36.8	21.2	21.2
Frozen savoy cabbage leaves[a]	372.0	293.0	292.0
Frozen lima bean leaves[a]	363.0	344.0	348.0
Frozen carrots and peas	94.0	...	95.5
Fresh spinach	87.8	76.5	74.3

[a] Results expressed on dry-weight basis.

These lower values agree very well with those obtained by the present method. The adsorption procedure not only removes chlorophyll and xanthophyll but also the less common pigments, such as lycopene and the noncarotene pigments of peppers, squash, and pumpkin. Samples that had been dehydrated and stored showed a considerably greater proportion of noncarotene pigments than dehydrated samples analyzed immediately. Samples stored for 3 to 6 months at room temperature lost large amounts of carotene, some undoubtedly being converted into noncarotene pigments, which are estimated as carotene by phasic procedures. Moore (*4*) and Fraps and Kemmerer (*1*) have presented similar results. The adsorption procedure also removes considerable noncarotene impurities from silage. On the whole the results of the phasic procedures show better agreement with those of the adsorption technique when fresh and frozen vegetable materials are used than on dried or ensiled samples.

A comparison of the values obtained by the magnesium oxide adsorbent with those obtained by the Baker's c. p. analyzed dicalcium phosphate adsorbent described by Moore (*4*) indicates that the adsorbents would give identical results. In many cases, however, it was necessary to readsorb the eluate that had passed through the dicalcium phosphate in order to remove small amounts of chlorophyll. In addition, the dicalcium phosphate at times may have to be specially activated in the laboratory. It is very susceptible to traces of polar solvents and much more expensive than the infusorial earth–magnesium oxide mixture, so that it is not so well adapted to routine procedures.

Duplicates run by the adsorption method agree within 3 to 5 per cent. It is difficult to estimate the accuracy of the determination, since a small and variable amount of alpha-carotene and possibly, with some materials, other carotene isomers are estimated as beta-carotene. Carotene-recovery tests and comparison with other extraction procedures indicate, however, that the adsorption procedure is considerably more accurate than phasic procedures in determining the carotene content of dehydrated and stored feeds or silage and the recovery of carotene by this method is of a high order. The authors have observed that the adsorbent will qualitatively separate alpha- and beta-carotene if the column is washed with pure Skellysolve B or Skellysolve B and 1 per cent or less acetone, and that carotene from yellow corn may easily be separated from cryptoxanthin by this procedure.

Summary

A rapid, reproducible method for the determination of carotene in plant materials, either dehydrated or fresh, has been described in detail. The method for dehydrated products consists essentially of extraction of carotene with a mixture of 30 per cent acetone and 70 per cent Skellysolve B, and subsequent separation of the carotene (alpha and beta) from interfering pigments on a column of three parts of Hyflo Super-Cel and one part of Micron Brand activated magnesium oxide. This adsorbent requires no special laboratory activation and does not destroy carotene. For dehydrated materials, phasic operations are completely eliminated, thus making the method much more rapid than any of the published phasic procedures. Fresh material is extracted by modification of the Moore and Ely method in which the Waring Blendor is used. A comparison of the results obtained by the method described with those obtained by the Peterson-Hughes-Freeman method indicates that considerable amounts of noncarotene pigments are estimated by procedures based on the phasic separation of carotene from noncarotene pigments.

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Studies in Filter Photometry

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THE single barrier layer photocell is one of the most widely used instruments of this nature, and has been subject to the most varied manner of empirical calibration. There is a definite need for improvement, particularly in respect to the exactness and fundamental nature of the measurements made and being reported with these instruments. Müller (*3*) has reviewed the literature of photoelectric photometers comprehensively; hence there is no need of considering this aspect further.

The most important use of filter photometers is in colorimetric analysis. When the solution obeys Beer's law, the relationship between log transmittance and concentration is linear. The need for instrument calibration in transmittance or its logarithm is obvious. Sheard and States (*6*) have summarized the situation existing with the use of barrier layer photocell photometers when they state that failure to obey Beer's law has been ascribed more frequently to the photocell than to the lack of monochromatic illumination. Experimental work was undertaken to determine the effect of the wave band of spectral illumination on the type of analytical calibration curve obtained. The colorimetric determination of silica by means of the silicomolybdic acid reaction was used as a test method and is particularly interesting because the absorption occurs in a spectral region of minimum sensitivity.

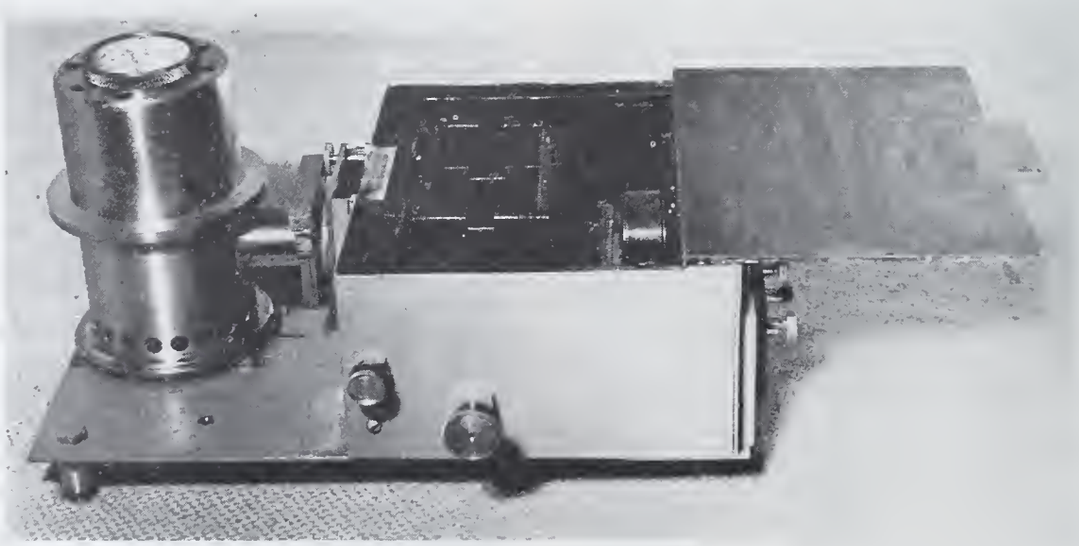

Figure 1. Filter Photometer

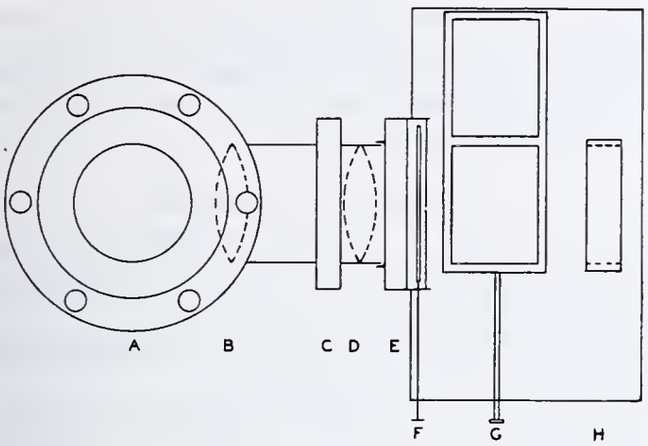

FIGURE 2. FILTER PHOTOMETER

A. Light
B. Lens
C. Heat-absorbing filter holder
D. Lens
E. Filter holder
F. Shutter
G. Absorption cell holder
H. Barrier layer photocell

Filter Photometer

The photometer consists of lamp and ventilated lamp housing to which is attached a collimating tube containing a lens for producing parallel rays of light, a holder for a heat-absorbing filter, and another lens for producing a very slightly divergent beam of light. A light-tight box with hinged cover contains a shutter and an external handle which moves it in and out of the path of light, a holder for two absorption vessels which may alternately be moved in and out of the path of the light by an external handle, and a barrier layer cell. On the outside of the box is the holder for filters. An air gap is provided between the two separate portions of the photometer but stray light is excluded by overlapping collimating tubes. The apparatus is pictured in Figure 1.

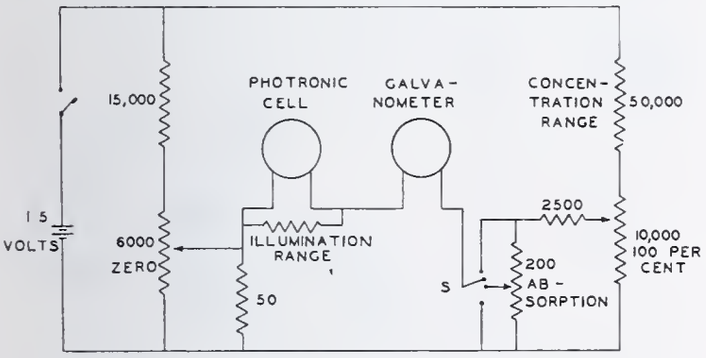

FIGURE 3. FILTER PHOTOMETER CIRCUIT

A schematic diagram of the filter photometer is represented in Figure 2 and the electrical circuit for obtaining potentiometric photocurrent balance is presented in Figure 3. The light source is a 32- or 50-candlepower, 6- to 8-volt lamp, the lenses are of the simple convex type, the heat-absorbing filter is Corning No. 397, the absorption cells are 50 × 50 × 50 mm. and 50 × 50 × 10 mm., the barrier layer cell is a Weston Model No. 594, Type 1 or Type 2, and the filters are of Corning glass. The measurements are actually made in terms of per cent absorption from which per cent transmittance is easily computed. Since the position of the lamp holder is adjustable, the light source can be easily changed. In addition to the lamps mentioned above a Westinghouse 85-watt mercury vapor lamp was used and a 110-volt, 100-watt prefocus lamp. A heavy-duty storage battery, trickle charger, G. M. galvanometer No. 2564-C, 1.5-volt dry cell, General Radio Co. variable resistors, and International Resistance Co. fixed resistors complete the list of apparatus.

In order to secure photocurrent from the photocell proportional to the light intensity it is necessary to use sufficiently low values of external circuit resistance. However, it is also true that when the light intensity and photocurrent are low, the linear response of the photocell is less affected by an increased value of the external resistance. In fact, if the product of external resistance times the current delivered—that is, the terminal potential of the barrier layer cell—is assigned an upper limit by the conditions of the experiment, then there exists assurance that the non-linearity will be below some fixed value. This is obtained in the circuit employed, using low values of illumination range resistance for high values of illumination (large photocell currents) and vice versa, because their product is limited by the magnitude of the balancing voltage across the potentiometer. Thus uniform characteristics are obtained for wide ranges of illumination. Experimental conditions were such that a linear response of the photocell to light intensity could be reasonably expected.

The stability and reproducibility of the filter photometer were evidenced not only by consistent readings over a period of several months but by securing reproducible values of transmittance even when changing the light intensity from that of 32- and 50-candlepower, 6-volt lamps to that of a 110-volt, 100-watt lamp and when changing the current and spectral sensitivity from those of Type 2 to Type 1 photronic cells. On several occasions the instrument was dismantled and reassembled; after alignment, the transmittance figures previously secured were reproduced.

Photometer Operation

1. The light source is illuminated by the 6-volt battery.
2. The 1.5-volt battery is turned on.
3. Distilled water is used for a reference solvent. With distilled water in the light beam and switch *S* in the zero absorption position, the galvanometer is adjusted to zero with the "zero" dial. If necessary, the illumination range resistance is changed to make this possible.
4. With switch *S* in the 100 per cent absorption position and the shutter now placed in the light beam, the galvanometer is adjusted to zero with the "100 per cent" dial. If necessary, the concentration range resistance is changed to make this possible.
5. 3 is rechecked.
6. With the unknown solution brought into the light beam and switch *S* in center or absorption position, the galvanometer is adjusted to zero with the absorption dial. The absorption dial is calibrated from 0 to 100 in 0.5 divisions and reads directly in per cent absorption. 100 − the dial reading equals per cent transmittance.

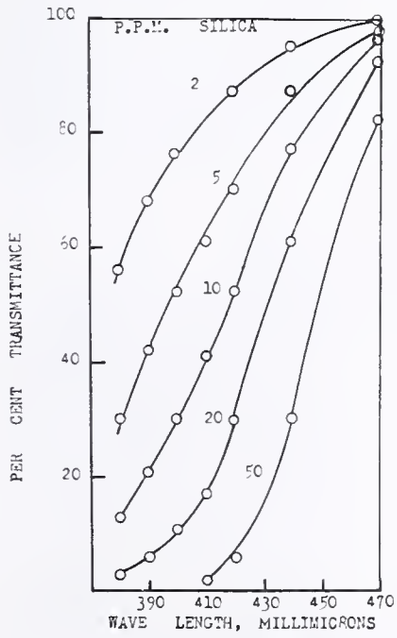

FIGURE 4. TRANSMITTANCE OF SILICOMOLYBDIC ACID

The two branches of the potentiometer circuit render it possible to make the full range of the 0–100 scale cover a narrower range of absorption than 0–100 if it is desirable to do so. The introduction of this feature necessarily complicates the circuit by the addition to the basic balancing circuit, in which the output voltage of the barrier layer cell across the illumination range resistor is balanced potentiometrically against a standardized drop, of another potential divider cir-

Table I. Transmittance

Concentration	Transmittance[a] Full range 0–100%	Full range 0–24%
P. p. m. SiO_2	%	%
2	83.5	78
5	64.5	53
10	44	26
15	32	10

[a] Filter photometer; filter combination No. 511, 4 mm., and No. 038, 2 mm.; 50-mm. liquid depth; silica from sodium silicate.

Table II. Transmittance of Silicomolybdic Acid

Concentration	Wave Length, Millimicrons 390	400	410	420	440	470
	Transmittance[a]					
P. p. m. SiO_2	%	%	%	%	%	%
2	68	76	82	87	95	99.5
5	42	52	61	70	87	97.5
10	21	30	41	52	77	96
20	6	11	17	30	61	92
50	..	0.5	2	6	30	82

[a] Coleman double monochromator spectrophotometer, 19-mm. liquid depth, SiO_2 from sodium silicate.

cuit to establish a "false" zero for the barrier layer cell when the equivalent scale range is "spread". This is done by using a standard solution instead of the shutter in step 4—for example, 20 p. p. m. of silica show an absorption of 24 per cent. With such a solution in place (more conveniently done by using a buffered potassium chromate liquid standard for silica) the full range of the absorption dial covers 0–24 rather than 0–100. Characteristic results of such a test are shown in Table I.

As a reference instrument a Coleman double monochromater spectrophotometer was used.

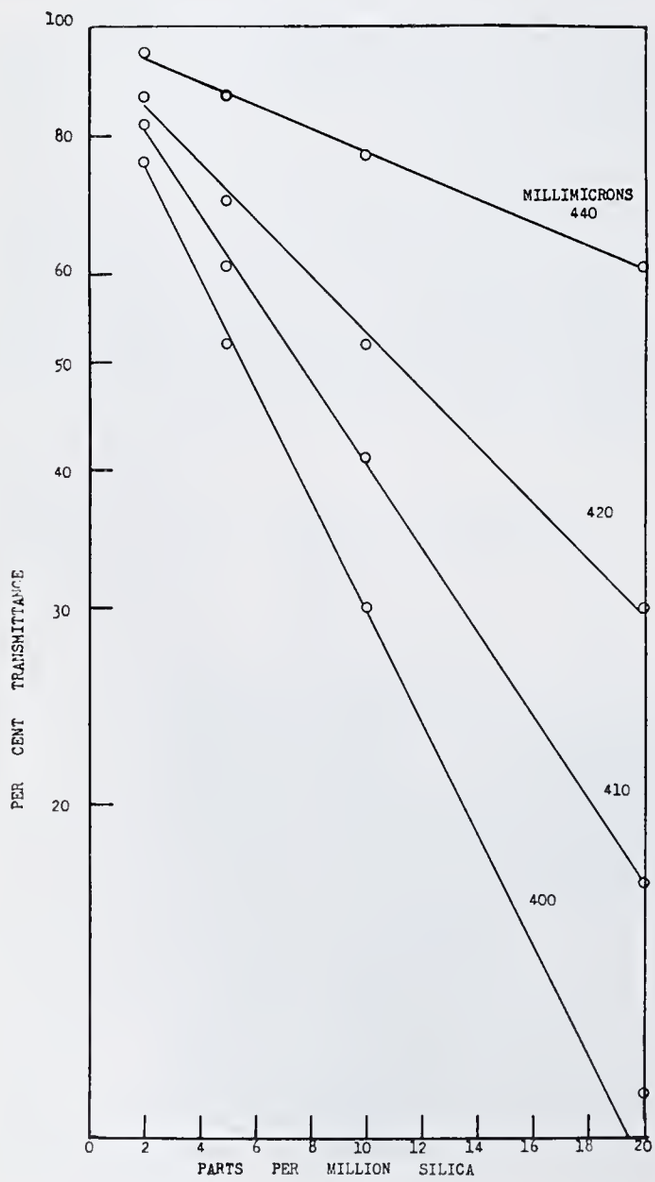

Figure 5

Reagents and Procedure

Ammonium molybdate solution, 10.0 grams of ammonium molybdate tetrahydrate per 100 ml. of distilled water.

Hydrochloric acid solution, one volume of concentrated acid to one volume of distilled water.

Sodium silicate solution, 3.0 grams of sodium carbonate fused with 0.2000 gram of pure, dry silica, dissolved in 200 ml. of distilled water, and diluted tenfold if desirable.

The ammonium molybdate and hydrochloric acid solutions are kept in Pyrex bottles; the sodium silicate solution is stored in a hard-rubber bottle.

To the 100-ml. sample are added and mixed 4 ml. of ammonium molybdate solution and 2 ml. of hydrochloric acid solution in rapid succession. It is advisable to wait 5 to 10 minutes for full color development.

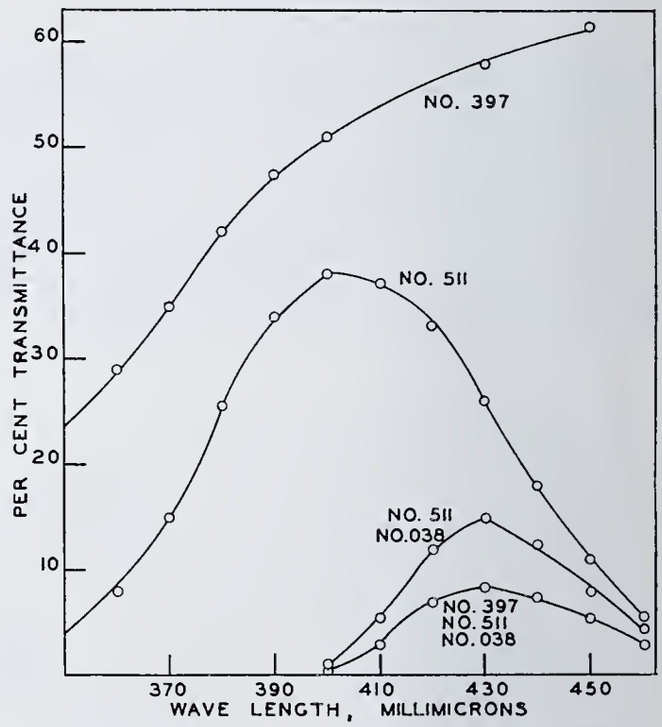

Figure 6. Spectral Transmission of Filters

As fundamental data the spectral transmittance of silicomolybdic acid, covering a reasonable range of concentration, was secured with the Coleman spectrophotometer (Table II and Figure 4). The data of Table II are replotted to give log per cent transmittance *vs.* concentration at constant wave length. As the data of Figure 5 show, the analytical linear calibration curves begin to deviate at wave lengths below 400 millimicrons. For maximum sensitivity it is preferable to work in the neighborhood of 410 millimicrons.

Several investigators using filter photometers have employed filters in this range of wave length. With the Pulfrich photometer for measuring instrument, Krumholz (*2*) used a 470 millimicron filter; Strohecker, Vaubel, and Breitwiesser (*7*) and Robinson and Spoor (*5*) used a 430 millimicron filter; while Pinsl (*4*) used a mercury vapor lamp and mercury (436) filter. Knudson, Juday, and Meloche (*1*) used the Corning No. 511, Cenco No. 1, and Evelyn No. 420 filters in a filter photometer. An examination of the Corning filter catalog indicated the use of filters 511 and 038. The data of Table

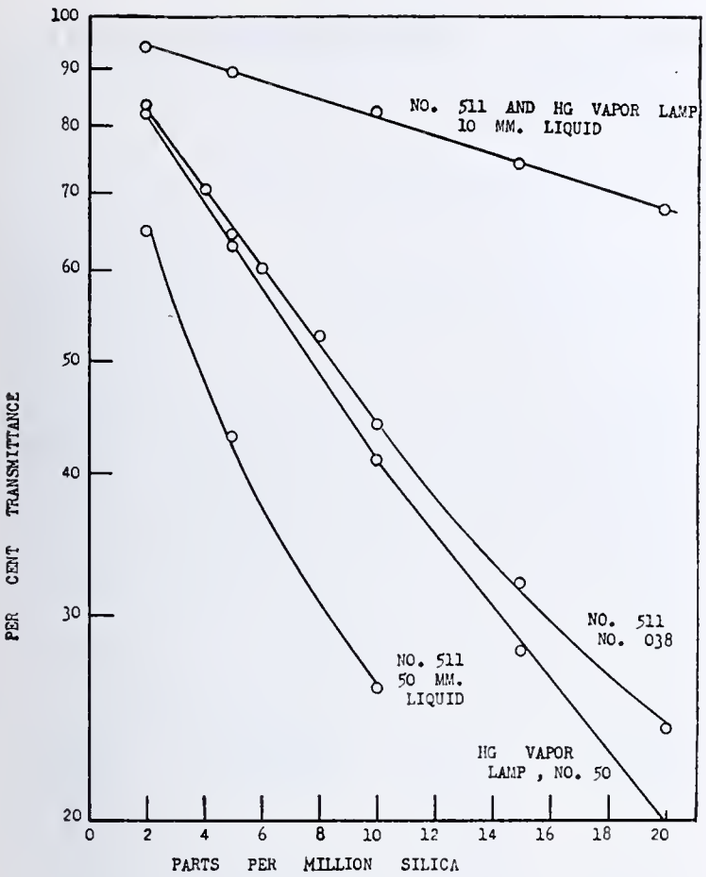

FIGURE 7

TABLE III. SPECTRAL TRANSMISSION OF FILTERS

Wave Length	Corning Filter Combinations			
	No. 511, 4 mm.	No. 511, 4 mm. No. 038, 2 mm.	No. 397, 2 mm.	No. 511, 4 mm. No. 038, 2 mm. No. 397, 2 mm.
Millimicrons	Transmission[a] %	%	%	%
350	4.0	..	23.5	..
360	8.0	..	29.0	..
370	15.0	..	35.0	..
380	25.5	..	42.0	..
390	34.0	0.0	47.5	..
400	38.0	1.0	51.0	0.5
410	37.0	5.5	54.0	3.0
420	33.0	12.0	..	7.0
430	26.0	15.0	58.0	8.5
440	18.0	12.5	..	7.5
450	11.0	8.0	61.5	5.5
460	5.5	4.5	..	3.0
500	..	..	67.0	..

[a] Coleman double monochromator spectrophotometer.

III and Figure 6 present the spectral transmission of these filters, including the heat-absorbing filter No. 397, taken with the Coleman spectrophotometer.

The transmittance of silicomolybdic acid solutions, through a reasonable range of concentration, was determined with the filter photometer using various combinations of filters and light sources (Table IV). The data of Table IV are likewise replotted to give log per cent transmittance *vs.* concentration at different filter combinations. The data presented in Figure 7 show in accordance with information secured by the spectrophotometer that the filter combination Nos. 511 and 038, giving as they do a narrow band of light in the proper spectral region, give closest approach to a linear analytical curve when using filters. Somewhat better agreement using the mercury vapor lamp and filter is in further accord with the need for wave-length selection. This effect and the resulting

TABLE IV. TRANSMITTANCE OF SILICOMOLYBDIC ACID

Concentration	Filter Combinations				
	No. 511, 4-mm., 10 mm. of liquid	No. 511, 4-mm., 50 mm. of liquid	No. 511, 4-mm. No. 038, 2-mm., 50 mm. of liquid	Hg Vapor Lamp No. 50 50 mm. of liquid	Hg Vapor Lamp No. 50 10 mm. of liquid
P. p. m. SiO_2	Transmittance[a] %	%	%	%	%
2	94	65	83.5	83	95
4	..	..	70.5	..	..
5	89	43	64.5	63	89.5
10	82	26	44	41	82
15	74	..	32	28	..
20	..	..	24	20	68
25	..	..	19	..	..

[a] Filter photometer, SiO_2 from sodium silicate.

deviation above 10 p. p. m. become more noticeable as the solution thickness and concentration increase. The data furnished by the investigation show that the controlling factor in obtaining linear analytical calibration curves with a filter photometer is in securing the proper wave band of light; when the spectral region is properly defined for a particular analysis and the more nearly the spectral region isolated approaches the ideal, the more sensitive and in general the more nearly linear the calibration curves become. The effectiveness of a spectrophotometer at once becomes obvious and the apparent failure of a solution to obey Beer's law as obtained on many filter photometers is no doubt due, in part anyway, to improper wave-length selection, particularly in using wide wave bands.

The calculated specific extinction coefficient per 1 cm. of liquid thickness and per 1 p. p. m. of silica for the 511–038 filter combination is 0.007 (0.006 to 0.008) as compared to 0.006 for the comparable 440 millimicron data on the Coleman spectrophotometer. Strohecker, Vaubel, and Breitwiesser (7) obtained an average value of 0.010, using, however, different reagent strengths for developing the color reaction. The specific extinction coefficient for the 410 millimicron data on the Coleman spectrophotometer is 0.021 (0.020 to 0.023).

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Quantitative Determination of Methoxyl and Ethoxyl Groups

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THE determination of alkoxyl groups is based on their conversion into volatile alkyl iodides on treatment of an alkoxyl-containing compound with constant-boiling hydriodic acid. The direct determination of total alkoxyl, or of methoxyl or ethoxyl alone, is accomplished very readily by the Zeisel procedure (*8*) as modified by Vieböck (*4, 5*) and by Friedrich (*2*). In this procedure the alkyl iodide, after passing through a scrubber solution of cadmium sulfate and sodium thiosulfate (to remove hydriodic acid) is oxidized to iodate in a bromine–glacial acetic acid–potassium acetate solution (*5*) according to the following reactions:

$$Br_2 + RI \rightarrow RIBr_2 \rightarrow RBr + IBr$$
$$IBr + 2Br_2 + 2H_2O \rightarrow HIO_3 + 5HBr$$

The resulting iodate is then determined iodometrically by conversion to free iodine, which is titrated with sodium thiosulfate.

In order to differentiate between methyl iodide and ethyl iodide, the difference in solubility of tetramethyl ammonium iodide and of trimethylethyl ammonium iodide in alcohol is employed.

In the method of Willstätter and Utzinger (*6*), the alkyl iodides are absorbed in a 10 per cent absolute alcoholic trimethylamine solution. Although the reaction between trimethylamine and methyl iodide is rapid (98 per cent complete in 1 hour), that with ethyl iodide is very much slower; in consequence, the absorption solution is allowed to stand for at least 24 hours before the trimethylethyl ammonium iodide is separated from the tetramethyl ammonium iodide by extraction of the mixture of iodides with absolute alcohol. Each of the iodides is then determined gravimetrically by conversion to silver iodide with aqueous silver nitrate. When Wilson (*7*), however, showed that 100 cc. of absolute alcohol dissolve 0.040 gram of tetramethyl ammonium iodide at 25° C., Phillips and Goss (*3*) modified the Willstätter procedure by applying a correction for the solubility of the tetramethyl salt in the alcohol. In the Phillips and Goss procedure 100- to 300-mg. samples of known mixtures of *p*-ethoxybenzoic acid and anisic acid were used.

In order to avoid the necessity of correcting for the solubility of the tetramethyl salt in absolute ethanol, Wilson suggested the use of a saturated solution of tetramethyl ammonium iodide in this solvent as the trimethylethyl salt extractant. This modification has been incorporated in a tentative A. O. A. C. method for the determination of methanol in distilled liquors (*1*).

There are several disadvantages in Willstätter's (*6*) procedure for the determination of methoxyl and ethoxyl groups even after the above modifications are included. In the first place, during the hydrolytic stage of the process, the tetramethyl ammonium iodide separates in the absorption tubes in the form of minute crystals which tend to adhere firmly to the side of the tubes or flasks. Such crystals must be carefully separated with a rubber policeman or fine feather; the removal of those crystals adhering to the inside of the small-bore delivery tube is particularly difficult. Secondly, in order to isolate the ethyl derivative it is necessary to allow the absorption solution to stand for at least 24 hours. Even after this period, it is generally found that the quantity of trimethylethyl ammonium iodide isolated is somewhat less than the theoretical value. Finally, the yield of the two iodides is determined gravimetrically, either directly as the quaternary ammonium iodide (*1*) or as silver iodide.

The present investigation was carried out with the idea of eliminating the tedious, time-consuming steps in this procedure and developing a simpler and more accurate method for the simultaneous determination of methoxyl and ethoxyl groups.

The first difficulty, that of the complete removal of the crystalline tetramethyl salt, was overcome by washing out the absorption tubes with a small quantity of water followed by stock 97 per cent ethanol. As a result of this innovation, the necessity for using an absolute ethanol solution of trimethylamine was also avoided, 95 to 97 per cent ethanol being found satisfactory. The addition of water necessitated the drying of the mixed quaternary ammonium iodides prior to the extraction of the ethyl salt, but this step presented no difficulty.

TABLE I. EFFICIENCY OF ABSORPTION OF METHYL IODIDE FROM VANILLIN

(10- to 15-mg. samples in 10% alcoholic trimethylamine)

No. of Absorption Tubes	Absorption Solution	Tetramethyl Salt Extracted with Absolute EtOH	OMe (Theoretical Value 20.4)
2	HOAc-Br_2	....	20.3
2	HOAc-Br_2	....	20.2
2	HOAc-Br_2	....	20.2
2	$(CH_3)_3N$	No	19.8
2	$(CH_3)_3N$	No	20.0
2	$(CH_3)_3N$	Yes	19.9
2	$(CH_3)_3N$	Yes	19.7
2	$(CH_3)_3N$	Yes	19.3
2	$(CH_3)_3N$	Yes	19.9
2	$(CH_3)_3N$	Yes	19.9
3	$(CH_3)_3N$	No	20.3
3	$(CH_3)_3N$	No	20.0
3	$(CH_3)_3N$	No	20.2
3	$(CH_3)_3N$	Yes	20.3
3	$(CH_3)_3N$	Yes	19.7
3	$(CH_3)_3N$	Yes	20.0
3	$(CH_3)_3N$	Yes	20.2

The weakest point in the present methods for a combined methoxyl-ethoxyl determination lies in the fact that it is difficult to isolate the ethyl iodide quantitatively. Willstätter (*6*) showed that the formation of trimethylethyl ammonium iodide is 9 per cent complete in one hour, 77 per cent in 6 hours, and only 90 per cent in 24 hours. In order to avoid this difficulty it was found that the determination could be run in less time and with greater accuracy by first determining total alkoxyl according to the standard Vieböck-modified Zeisel procedure, then determining the methoxyl content alone according to the modified Willstätter procedure described below.

In order to simplify the actual determination of the tetramethyl ammonium iodide the gravimetric method was replaced by a volumetric method, using the same reagents and procedure employed in the total alkoxyl determination according to Vieböck (*4, 5*).

Procedure

METHOXYL DETERMINATION. A sample of 10 to 20 mg. (having from 10 to 40 per cent alkoxyl calculated as methoxyl) in a tin-foil microcontainer is added to the reaction vessel of the Zeisel apparatus containing the standard reaction mixture of constant-boiling hydriodic acid (5 cc.), phenol (2.5 grams), and a small piece of Nichrome wire. The procedure for this phase of the determination duplicates that of the standard Vieböck-Schwappach except for the substitution of a total of 15 cc. of a 10 per cent ethanol solution of trimethylamine as the absorption solution in place of the acetic acid–potassium acetate solution of bromine, and the use of three test tubes as receivers (the 15 cc. of absorption solution being divided into 6-cc., 5-cc., and 4-cc. portions, respectively).

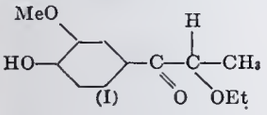
At the end of a reflux period of 50 minutes the liquid contents of the absorption tubes are transferred to a 50-cc. Erlenmeyer flask with ethanol (95 to 100 per cent). The crystalline residues left in the first and second absorption tubes are then washed with 2-cc. portions of water to dissolve the crystals of tetramethyl ammonium iodide adhering to the glass, then with two subsequent portions of ethanol. The combined absorption solutions plus washings are allowed to stand for at least 3 hours at room temperature. A small boiling chip of porous plate is then added to the flask and the contents are evaporated almost to dryness on a steam bath, or by use of a hot plate (if carefully watched to prevent complete evaporation with resulting decomposition of the quaternary iodides). Complete drying is effected by leaving for 12 hours in a vacuum desiccator containing calcium chloride. The trimethylethyl ammonium iodide is then extracted as follows:

A 3-cc. portion of a filtered, saturated solution of tetramethyl ammonium iodide in absolute ethanol is added to the flask by means of a medicine dropper pipet. (If absolute ethanol alone is used as extractant, a correction of 0.2 cc. of thiosulfate for 9 cc. of ethanol extractant must be added to the quantity of thiosulfate used in the titration.) After the mixture has been shaken gently for about 1 minute, the crystalline product is allowed to settle on one side of the tilted flask and the supernatant liquor is transferred to a glass-nail suction filter by means of a second pipet. This treatment is repeated twice. After the final decantation the transfer pipet is dried free from ethanol in a slow current of air, and the traces of tetramethyl compound are dissolved in distilled water. The aqueous solution is placed in a second flask, which also contains the air-dried filter paper. The residual contents of the original Erlenmeyer are freed from traces of ethanol in a vacuum desiccator. After 1 to 2 hours of this vacuum treatment, the aqueous contents of the second flask are transferred to the original flask, and the aqueous solution of tetramethyl ammonium iodide is then ready for volumetric determination.

The aqueous solution of the quaternary ammonium iodide is treated with 10 cc. of a saturated glacial acetic acid solution of potassium acetate containing 8 to 10 drops of bromine for 2 to 3 minutes, followed by the addition of 5 cc. of 25 per cent aqueous sodium acetate. The bromine is reduced with 10 drops of formic acid (80 to 90 per cent) and the resulting clear solution then treated with 5 cc. of 10 per cent aqueous potassium iodide solution and 5 cc. of 10 per cent sulfuric acid. The liberated iodine is titrated with standard (0.05 N) thiosulfate, using starch solution as an internal indicator.

Table II. Methoxyl-Ethoxyl Analyses

Compound	Found OMe	Found OEt	Calculated OMe	Calculated OEt
Vanillin	20.2	0.1	20.4	0.0
Vanillin	20.1	0.2	—	—
Vanillin	20.3	0.0	—	—
p-Ethoxybenzoic acid	0.0	27.0	0.0	27.2
p-Ethoxybenzoic acid	0.0	27.2	—	—
3-Ethoxy-4-methoxybenzoic acid	15.8	23.0	15.8	23.0
3-Ethoxy-4-methoxybenzoic acid	15.7	22.9	—	—
3-Ethoxy-4-methoxybenzoic acid	15.9	23.1	—	—
3-Ethoxy-4-methoxybenzoic acid	15.8	23.0	—	—
3-Ethoxy-4-methoxybenzoic acid	16.0	23.2	—	—
3-Ethoxy-4-methoxybenzoic ocid	15.9	23.1	—	—
3-Ethoxy-4-methoxybenzoic acid	16.0	23.2	—	—
3-Ethoxy-4-methoxybenzoic acid	15.7	22.9	—	—
α-Ethoxypropiovanillone (I)	13.7	20.2	13.8	20.1
	13.6	20.3	—	—
	13.7	20.2	—	—



The methoxyl and ethoxyl contents are calculated according to the following equations:

$$\% \text{ OMe} = \frac{(\text{cc. of thiosulfate}) \times (\text{normality of thiosulfate}) \times 31}{6 \times (\text{sample weight})}$$

$$\% \text{ OEt} = \left[\frac{(\% \text{ total alkoxyl as OMe}) - (\% \text{ OMe})}{31}\right] \times 45$$

Discussion of Results

Table I indicates that all the methyl iodide is collected when three receivers are used and that the yield of tetramethyl ammonium iodide is not affected by the ethanol extraction.

Table II includes the results obtained by this procedure with certain reference compounds.

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Recovery of Solvents Used in the Chemical Determination of Thiamine

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WHERE routine thiamine determinations are run by either the thiochrome method (*1*) or the colorimetric procedure (*2*) it is practical, and at the present time advisable, to recover the solvents, isobutanol in the former case and xylene in the latter. The distillation procedure generally used is tedious and requires attention; precautions must be observed to minimize fire hazards, and fluorescent materials in the isobutanol are not always completely removed.

The author has obtained isobutanol with blank readings much lower than those usually obtained by distillation in an all-glass still by treating waste solvent, containing thiochrome and other fluorescent materials, with activated vegetable charcoal (Darco-G-60, obtained from the Darco Corporation, 60 East 42nd St., New York, N. Y.). The isobutanol recovered in this manner has been used repeatedly in determining thiamine in a large variety of biological materials. Technical grade isobutanol, which usually contains fluorescent substances, may also be treated in this manner. The recovered isobutanol has proved to be as satisfactory in every respect as the solvent redistilled in all-glass apparatus; it fails to give rise to fluorescent substances on standing or after treatment with alkaline potassium ferricyanide or with alkali alone, according to the procedure for the estimation of thiamine.

The isobutanol, previously dried in the course of the assay, is recovered by shaking 100 cc. of solvent with 1.5 grams of activated charcoal for 15 to 30 minutes. This ratio of adsorbent to solvent is optimal, but equally good results are obtained by using an excess of charcoal—about 5 grams per 100 cc.—and shaking for only a few minutes. The charcoal suspension is then clarified by filtration. The filtrate, free from any fine particles of adsorbent, is used without further treatment.

In the Melnick-Field method for the determination of thiamine (*2*) the red color of the xylene solution is subjected to colorimetric evaluation. Here also, the solvent may be decolorized by the addition of activated charcoal, filtered, and used again.

The red dye complex in xylene appears to be adsorbed to the charcoal even more readily than the thiochrome from isobutanol solution. About 1 gram of charcoal is added to 100 cc. of the pigmented xylene solution and the suspension shaken for 5 minutes; this yields a colorless filtrate.

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Separation of Iron from Cobalt or Nickel

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THE ammoniacal ferricyanide method for the colorimetric determination of cobalt (*2*) requires the absence of practically all other metals. Using hydrogen sulfide, it is a simple matter to obtain the cobalt accompanied by only iron, nickel, manganese, chromium, aluminum, and zinc. The latter three may be removed by treatment with sodium peroxide, and the manganese by the use of the chlorate method (*4*). Nickel may be separated with dimethylglyoxime (*8*), but since the presence of iron requires careful control of the pH in this precipitation, a method was sought whereby either cobalt or nickel could be freed of iron, and a cobalt solution would be obtained ready for the colorimetric determination without the necessity of an intermediate reprecipitation of cobalt sulfide. With such a method of separation, the colorimetric method for cobalt could be easily applicable to steels, as well as to paint driers.

Experimental

In order to investigate published methods of separating mixtures of cobalt, iron, nickel, and manganese, solutions containing known amounts of these metals were prepared using Baker's C. P. chemicals. Methods of separating mixtures of all these metals were studied, but since it soon was evident that manganese and nickel could be separated quantitatively by accepted methods (*4, 8*), further work was concentrated on mixtures of cobalt and iron.

Ammonium Hydroxide Precipitation. A solution containing 2 mg. of cobalt and 20 mg. of iron was subjected to successive reprecipitations with ammonium hydroxide. The filtrates were analyzed colorimetrically for cobalt (*2*), and the precipitates were tested by the Vogel test (*6*) for cobalt, yielding the following data:

	Cobalt	Vogel Test
	Mg.	
Original	2.0	.....
1st precipitation	0.7	Positive
2nd precipitation	0.45	Positive
3rd precipitation	0.00	Positive

Only 57.5 per cent of the original cobalt was recovered in the combined filtrates.

Basic Acetate Separation. This method (*7*) was tried several times. Only once was the full amount of cobalt recovered, and the iron precipitate gave a negative Vogel test. Hence, though this method may be feasible under ideal conditions, it cannot be trusted for routine work.

Anthranilic Acid Separation. This method (*3*) was tried, again on the mixture of 2 mg. of cobalt and 20 mg. of iron. The precipitate was strongly contaminated with iron.

Alpha-nitroso-beta-naphthol (*1*) Separation. Since this reagent may precipitate iron as well as cobalt, it was not considered further.

Hydrochloric Acid Separation. In the qualitative analysis of Group III, cobalt and nickel sulfides are separated from the other sulfides and hydroxides by treatment with 1.2 *N* hydrochloric acid. However, no combination of time, temperature, or strength of hydrochloric acid could be found which dissolved all of the iron and none of the cobalt, for all ratios of these metals.

Oxalic Acid Separation. As ammonium hydroxide is added slowly to a solution of cobalt sulfate, a precipitate of cobalt hydroxide forms, then dissolves in additional reagent. If the solution is boiled, enough ammonia can be expelled to permit a reprecipitation of the hydroxide. Thus, the contamination by cobalt of the ferric hydroxide obtained by ammonium hydroxide precipitation may be due to coprecipitation rather than occlusion.

If oxalates are present with the cobalt sulfate, slow addition of ammonium hydroxide does not form a precipitate at any time. Even after prolonged boiling precipitation still does not occur. Hence coprecipitation could be prevented by the formation of a cobalt oxalate complex.

Then 2.0 mg. of cobalt as the sulfate, with the amounts of iron shown below as the chloride, were diluted to 200 ml. and 10 ml. of a 10 per cent solution of oxalic acid were added. The iron was precipitated with ammonium hydroxide as usual and filtered. The filtrate was analyzed colorimetrically (*2*) and the precipitate tested for cobalt by the Vogel test.

Iron	Cobalt in Filtrate	Vogel Test
Mg.	*Mg.*	
2.0	2.00	Negative
10.0	2.00	Negative
20.0	2.00	Negative
50.0	1.45	Positive
2000	0.30	Positive

To determine the solubility of iron in the ammoniacal oxalate solution, 20 mg. of iron as the chloride were carried through the same procedure in the absence of cobalt. The filtrate was boiled down, the oxalate destroyed with sulfuric acid, and the residue analyzed for iron (*5*); 0.18 mg. were found. This quantity of iron does not interfere with the colorimetric determination of cobalt (*2*).

The use of 5 ml. of the oxalic acid solution gave poor separation in all cases. The use of 20 ml. of oxalic acid did not improve the separation. The maximum ratio of iron to cobalt which can be separated quantitatively by this method is shown to be 10 to 1. An alloy steel containing 15 per cent of cobalt could be analyzed directly by this means, but one containing 0.1 per cent could not be so treated.

In order to obtain the required ratio of iron and cobalt the ether-extraction method for iron chloride (*9*) was employed. A solution of 2.0 mg. of cobalt and 2000 mg. of iron in hydrochloric acid was so treated. This removed about 95 per cent of the iron, leaving 100 mg. A second ether extraction reduced the iron to about 10 mg. The iron-cobalt ratio then was 5 to 1, so the oxalic acid method was applied. The filtrate was found to contain 2.00 mg. of cobalt.

In the above work, the oxalic acid was found not to interfere with the colorimetric method used for the cobalt, except that crystallization occurred during the removal of volatile anions.

Description of Method

Reagents: A 10 per cent solution of oxalic acid and concentrated (15 *N*) ammonium hydroxide.

The sample should contain from 0.5 to 4 mg. of cobalt. If the ratio of iron to cobalt is greater than 10 to 1, successive ether extractions of the chloride (*9*) must be made until the ratio is reduced to 10 to 1.

To the acid solution containing cobalt and iron, in a volume of about 200 ml., 10 ml. of oxalic acid solution are added. The solution is neutralized with ammonium hydroxide, and 5 ml. excess are added. The precipitate is coagulated by boiling, then filtered and washed. All the cobalt will be in the filtrate.

If the cobalt is to be determined colorimetrically, the salt concentration must be controlled (*2*). To accomplish this, 6 ml. of 9 *N* sulfuric acid are added to the acid solution of cobalt and iron, and volatile anions are removed by evaporating to fumes of sulfur trioxide. The solution is cooled, diluted, and neutralized (litmus paper) with sodium hydroxide, then made just acid with sulfuric acid, and the volume is brought to about 200 ml. Then 10 ml. of oxalic acid are added, and the iron is precipitated with ammonium hydroxide, as described above. After filtration, the volume is reduced to 60 ml., during which the excess ammonia is expelled. The solution is then ready for the addition of the 10 ml. of potassium ferricyanide and 20 ml. of ammonium hydroxide required for the cobalt determination. The oxalic acid does not interfere.

Separation of Nickel and Manganese

A solution of nickel sulfate was analyzed by the use of dimethylglyoxime. Mixtures of this solution and the iron solution, with a ratio of iron to nickel of 10 to 1, were prepared. The nickel was precipitated from these mixtures with dimethylglyoxime, with careful pH control (*8*). The iron was precipitated by the above oxalic acid procedure, and the nickel in the filtrate precipitated with dimethylglyoxime, without careful pH control. The results of the two methods were identical. Ratios of iron to nickel greater than 10 to 1 were not investigated.

Mixtures of nickel, cobalt, and iron were prepared, and the iron was precipitated by the above procedure. The nickel in the

filtrate was precipitated as before, and quantitative recovery obtained. The cobalt then was determined colorimetrically. If this was attempted directly on the filtrate, the dimethylglyoxime was found to interfere with the color development. By precipitating the cobalt as sulfide, filtering, and then analyzing colorimetrically, however, quantitative recovery was proved.

Mixtures of nickel, manganese, iron, and cobalt were made and the iron was removed as described. The manganese divided between precipitate and filtrate. That in the precipitate did not interfere with the colorimetric determination of the iron. The nickel was removed from the filtrate with dimethylglyoxime, then the manganese with chlorate (*4*), and finally the cobalt was freed of all these reagents by precipitation as the sulfide, and determined colorimetrically. Again, quantitative recovery was obtained.

These experiments indicate that the separation of iron from cobalt or nickel by the use of oxalic acid is not interfered with by the presence of any other element normally present at that point in the analysis.

Summary

By the addition of oxalic acid before precipitation of ferric hydroxide by ammonium hydroxide, coprecipitation of cobalt and/or nickel is prevented, and a quantitative separation of these metals can be obtained.

The oxalic acid does not interfere with the subsequent colorimetric determination of either the cobalt or the iron, or the quantitative determination of nickel.

By this method, as little as 0.1 per cent of cobalt may be determined in a steel.

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Determination of Aliphatic Nitrate Esters

A Colorimetric Method

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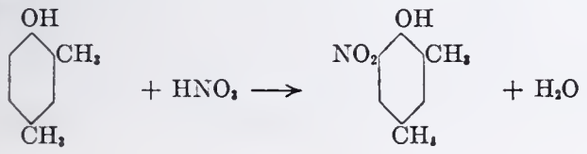
THE methods generally employed in the analysis of aliphatic nitrate esters are based on the estimation of the quantity of nitric or nitrous acid formed on the hydrolysis of the ester (*3*). While these methods are serviceable in the assay of small quantities of relatively pure ester material, it was not found practical to adapt the standard procedures to the analysis of biological samples containing nitroglycerine, erythritol tetranitrate, or pentaerythritol tetranitrate. Ethereal extracts of such samples are accompanied by other ether-soluble components such as fats and pigments which interfere with the colorimetry.

The method for the determination of inorganic nitrates described by Blom and Treschow (*1*), based on the formation of 5-nitro-4-hydroxy-1, 3-dimethylbenzene, seemed a practical approach to the solution of this problem, as the chromogenic compound is readily isolated from other substances interfering with the colorimetry by means of a steam-distillation. Experiments demonstrated that in the case of the three esters employed in the physiological studies, complete hydrolysis could be effected at room temperature by the action of 62.5 per cent sulfuric acid, and that the nitric acid formed could be determined quantitatively by the simultaneous nitration of *m*-xylenol. Subsequent experiments proved that the reactions proceeded in exact stoichiometric proportions and that the amount of ester present could be evaluated with the aid of an inorganic nitrate standard.

The reaction between nitric acid and the *m*-xylenol employed as reagent in sulfuric acid media is as follows:



The nitration product is readily volatile in steam and reacts with alkalies, forming an intense yellow-colored solution that obeys Beer's law. This reaction was utilized by Treschow and Gabrielsen (*5*) in the analysis of plant and soil extracts, and as a means of estimating inorganic nitrates in meat products by McVey (*2*). The sensitivity of the reaction was investigated by Werr (*6*), who found that in the absence of materials that interfered with the nitration process, 1 microgram of inorganic nitrate could be detected at a dilution of 1 part per million of sample solution.

The substances interfering with the reaction are large amounts of halogen salts, metals that liberate hydrogen with the sulfuric acid medium, nitrites, sulfides, and hydrogen peroxide. From the viewpoint of the proposed method for the analysis of nitrate esters, inorganic nitrates also constitute an interference. In the application of the method these interfering substances are of little importance, as the nitrate esters can be separated from them by an ether extraction. Traces of chlorides and inorganic nitrates which may dissolve in the ether layer are readily removed by washing the extract with 10 per cent sodium sulfate. Hydrogen peroxide will also dissolve in the ether layer, but its occurrence in appreciable quantity is not likely in samples of biological origin. When an extraction process is not employed, any sulfides or halogen salts present in the aqueous sample can be removed by precipitation with silver sulfate. Also, the traces of nitrite present in blood and urine would be destroyed in sulfuric acid media by the action of the urea and amino acids normally present in such samples (*4*).

Method of Analysis

Reagents. Sulfuric acid 62.5 per cent by volume, (sp. gr. 1.63) prepared by mixing 5 volumes of nitrate-free concentrated sulfuric acid with 3 volumes of water.

m-Xylenol, 2 per cent (Eastman preparation No. 1150, 4-hydroxy-1,3-dimethylbenzene) dissolved in acetone.

Sodium hydroxide solution, 2 per cent.

Potassium nitrate, 0.02 per cent.

The nitrate esters employed in the development of the method were of commercial origin. The erythritol tetranitrate and the pentaerythritol tetranitrate were purified by dissolving the compounds in warm acetone, filtering off insoluble matter, and diluting the filtrates with water at 50° C. The granular precipitates were filtered, washed with a mixture of acetone and water (1 + 3 by volume), and dried over calcium chloride in a vacuum desiccator. The nitroglycerine was not purified, but the commercial 10 per cent acetonic solution was assayed by evaporating off the solvent at room temperature and drying the residue to constant weight in a vacuum desiccator. Acetonic solutions of these preparations containing known amounts of the esters were employed in the development of the method.

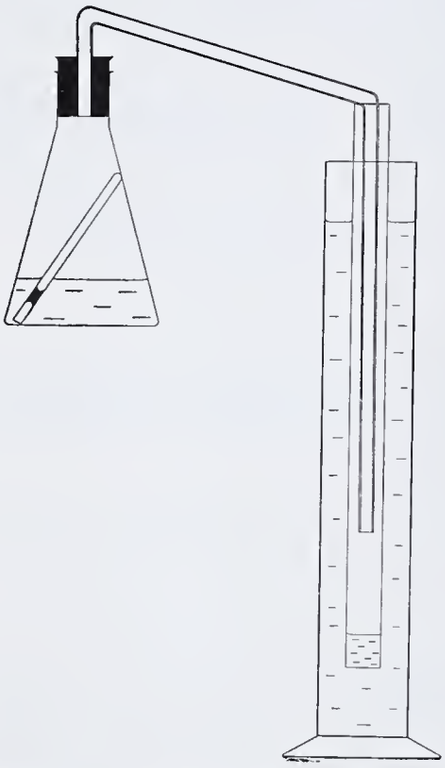


Figure 1. Apparatus for Distillation of Nitroxylenol

Preparation of the Sample. The sample of blood, urine, or stomach contents is extracted with ether and the extract washed with 10 per cent sodium sulfate until the wash liquor gives a negative chloride test with silver nitrate. The ether layer is transferred to a volumetric flask and an aliquot portion containing not more than 3 mg. of the ester is withdrawn to a 300-ml. cone flask. The ether is evaporated off with the aid of a gentle stream of cold air. Warming of the solvent and prolonged aeration are to be avoided, as the esters, particularly nitroglycerine, are easily volatilized. The residue need not be perfectly dry and the aeration should be stopped when the bulk of the ether has volatilized. The residue is treated with 1 ml. of a 2 per cent acetonic solution of *m*-xylenol. The use of the acetone as a solvent for the *m*-xylenol is of fundamental importance, as it redissolves the ester and facilitates the quantitative progress of the nitration. This is of particular importance in the case of pentaerythritol tetranitrate which is extremely insoluble in water and gives low results in the absence of the acetone solvent. The solution is then treated with 15 ml. of 62.5 per cent sulfuric acid and allowed to react at room temperature for 30 minutes. At the termination of this period the mixture is diluted with 100 ml. of water and the nitroxylenol is separated by distillation.

Distillation. If the distillation is conducted with an efficient water-cooled condenser, the nitroxylenol frequently solidifies on the condenser tube. This difficulty is overcome by conducting the steam directly into a water-cooled Nessler tube (50-ml., A.P.H.A. tall-form), as shown in Figure 1. The delivery tube is constructed so that the terminus is slightly above a 20-ml. calibration mark on the Nessler tube when the latter is buoyed up against the inclined arm. In the presence of fatty matter it is desirable to place a boiling rod and some porous tile in the flask to minimize bumping. The steam is collected in 5 ml. of 2 per cent sodium hydroxide, introduced into the tube so that the walls are wetted by the alkali. When the solution comes to a boil, the flame is reduced to a height of 2 to 3 cm. and 15 ml. of distillate are collected. The delivery tube is separated from the flask, and the lower arm is rinsed, allowing the wash water to run into the Nessler tube. The tube is cooled to room temperature, diluted to the 50-ml. mark, and the contents are mixed. The resultant yellow color is read in a Duboscq colorimeter, using 5-cm. cups against a standard solution of nitroxylenol prepared from a known weight of potassium nitrate.

Quantities of the esters over the range of 3.0 to 0.1 mg. can be matched against a standard prepared from 1 mg. of potassium nitrate. The standard is prepared by evaporating 5 ml. of 0.02 per cent potassium nitrate to near dryness, cooling, and reacting the residue with 1 ml. of the *m*-xylenol reagent and 15 ml. of 62.5 per cent sulfuric acid. This mixture is distilled after 30 minutes, following the same procedure as in the case of the samples. One milligram of potassium nitrate is equivalent to 0.7486 mg. of nitroglycerine, 0.7470 mg. of erythritol tetranitrate, and 0.7817 mg. of pentaerythritol tetranitrate.

Table I. Determination of Nitroglycerine

Potassium Nitrate Reading[a]	Nitroglycerine Taken	Nitroglycerine Recovered	Difference
Mg.	*Mg.*	*Mg.*	*Mg.*
3.81	3.00	2.85	−0.15
2.70	2.00	2.02	+0.02
1.26	1.00	0.94	−0.06
0.65	0.50	0.49	−0.01
0.26	0.20	0.19	−0.01
0.19	0.10	0.14	+0.04

[a] 1 Mg. of KNO_3 = 0.7486 mg. of $C_3H_5(NO_3)_3$.

Table II. Determination of Erythritol Tetranitrate

Potassium Nitrate Reading[a]	Erythritol Tetranitrate Taken	Erythritol Tetranitrate Recovered	Difference
Mg.	*Mg.*	*Mg.*	*Mg.*
3.88	3.00	2.90	−0.10
2.70	2.00	2.02	+0.02
1.30	1.00	0.97	−0.03
0.70	0.50	0.52	+0.02
0.23	0.20	0.17	−0.03
0.14	0.10	0.10	0.00

[a] 1 mg. of KNO_3 = 0.7470 mg. of $(CHNO_3CH_2NO_3)_2$.

Table III. Determination of Pentaerythritol Tetranitrate

Potassium Nitrate Reading[a]	Pentaerythritol Tetranitrate Taken	Pentaerythritol Tetranitrate Recovered	Difference
Mg.	*Mg.*	*Mg.*	*Mg.*
3.90	3.00	3.04	+0.04
2.56	2.00	2.00	0.00
1.50	1.20	1.17	−0.03
1.30	1.00	1.02	+0.02
0.85	0.60	0.66	+0.06
0.24	0.20	0.19	−0.01

[a] 1 mg. KNO_3 = 0.7817 mg. of $C(CH_2NO_3)_4$.

The results obtained in the analysis of known quantities of pure nitrate esters are summarized in Tables I to III. The data reveal that the reaction proceeds in stoichiometric proportions within the limit of error of visual colorimetry. The average reproducibility of results in the analysis of pure ester is 1 per cent. In the application of the method to ether extracts of the digestive tracts of animals fed the esters in question, analyses of successive aliquots are reproducible within 3 per cent. The results of these physiological studies will be published in another article.

By viewing the color of the sodium salt of the nitroxylenol through the 23-cm. length of the 50-ml. Nessler tube it is possible to differentiate 5 micrograms of the esters from the straw-colored tint of the blank on the reagents. The range of 0.1 to 0.005 mg. can thus be covered by the Nesslerization process. Since the tint of the blank is caused by the color of excess *m*-xylenol, it is advantageous to reduce the concentration of the *m*-xylenol in the acetone solution to 1 per cent when analyzing for traces of the esters. This procedure

has been employed in measuring the solubility of the aliphatic nitrate esters in water and oil. It should prove useful in the analysis of air samples from the atmosphere of plants engaged in the manufacture of the esters.

Summary

A colorimetric method for the determination of aliphatic nitrate esters is described, based on the hydrolysis of the ester in 62.5 per cent sulfuric acid and the nitration of *m*-xylenol by the nitric acid liberated. The method has been applied to nitroglycerine, erythritol tetranitrate, and pentaerythritol tetranitrate over a range of 3.0 to 0.005 mg. The nitroxylenol is readily volatilized by a steam-distillation which permits the application of the method to complex systems. The reaction proceeds in stoichiometric proportions, so that the quantity of ester present can be evaluated from a potassium nitrate standard.

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Determining Free and Acetylated Sulfanilamide

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THE most commonly used procedure of analysis for sulfanilamide in solutions where both the free and acetylated forms are likely to be present is that of Bratton and Marshall (*2*). This method permits a fairly rapid determination of sulfanilamide, but in order to determine the total amount of sulfa drugs present, it is necessary to hydrolyze the acetyl component by heating in boiling water for an hour. This becomes rather tedious when only one sample is to be analyzed.

During a recent investigation in this laboratory it became necessary to determine the ultraviolet absorption spectrum of sulfanilamide. The results obtained indicated that the difference in the absorption spectra of the two forms of the drug could be utilized in performing a rapid analysis of solutions where both free and acetylated sulfanilamide were likely to be present. With this in mind, the extinction coefficients of the two compounds were determined and an apparatus was constructed for making the analysis of aqueous solutions. The results are presented in this paper, together with a summary of certain modifications that will be necessary if attempts are made to adapt the method for use in an instrument to determine the concentration of sulfanilamide in the blood.

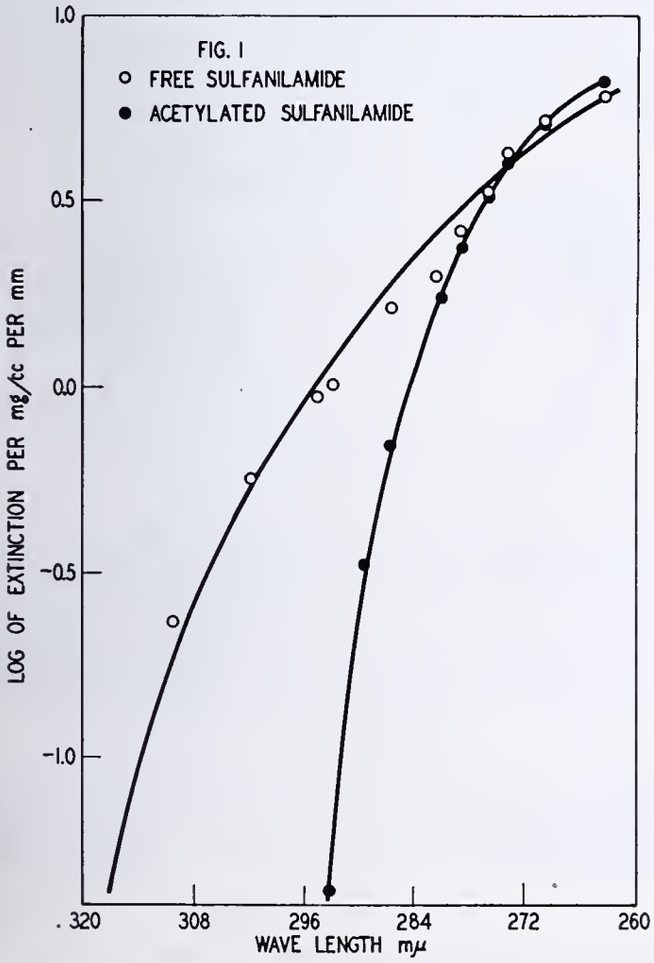

FIGURE 1. VARIATION OF EXTINCTION COEFFICIENT WITH WAVE LENGTH

Ultraviolet Absorption Spectra

The extinction coefficients of both free and acetylated sulfanilamide have been measured photographically in the spectral range 320 to 260 mμ.

For this work a Cenco replica grating spectrograph was employed, taking the spectra on Eastman Panchromatic process film. A hydrogen arc of the Allen (*1*) type furnished a source of an ultraviolet continuum. The absorption spectra were determined with solutions varying in concentration from 0.2 to 1.25 $\times 10^{-3}$ mg. per cc. These were placed in an absorption cell 13.7 mm. long equipped with Vycor windows. A step plate was added for calibration, and relative light intensities were determined with the aid of a Leeds & Northrup Speedomax recording microphotometer. Following Beer's law, $(\log I/I_0)/L$ was plotted against the concentration for a number of wave lengths in the region investigated. The negative slope of the resultant line gave the specific extinction in units of mg. per cc. per mm. when the concentration was expressed in mg. per cc. and the cell length, L, in millimeters.

A plot of the extinction coefficients as a function of wave length is shown in Figure 1 and the transmission spectra of the two compounds appear in Figure 2. Here spectrum 5 is for free sulfanilamide and spectrum 3 is for the acetylated form. From these we see that free sulfanilamide absorbs all light of wave lengths shorter than 310 mμ, while the acetyl derivative absorbs only wave lengths less than 286 mμ. Furthermore, the two compounds possess almost equal extinction coefficients for light in the range 282 to 260 mμ.

Since a difference exists between the spectra of the two forms of sulfanilamide, it was felt that this characteristic might be utilized in a rapid determination of the two compounds in solutions where both might be present together. This has been done for aqueous solutions. There should be no difficulty in extending the method to the corresponding analysis in blood solutions, provided certain necessary precautions are taken.

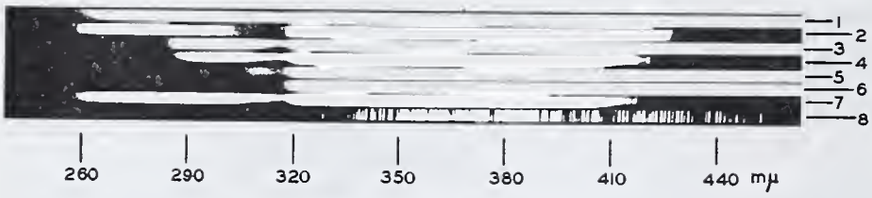

FIGURE 2. TRANSMISSION SPECTRA

Made with Cenco replica grating spectrograph (absorption occurring at 316 mμ in all spectra is due to transmission of silver coating on grating) using Eastman Panchromatic process film. Hydrogen arc used for ultraviolet continuum.

1. Transmission of water in Vycor Cell (limited in ultraviolet here by absorption of grating)
2. Transmission of Corning 9860 plus Corning 9840
3. Transmission of acetylated sulfanilamide
4. Transmission of Corning 9860 plus 0.125 inch of benzene
5. Transmission of sulfanilamide
6. Transmission of Corning 014
7. Transmission of Corning 9860
8. Brass arc

TABLE I. RECOVERY OF KNOWN AMOUNTS OF SULFA DRUGS IN WATER

Solution No.	Composition of Synthetic Solution: Sulfanilamide	Acetylated sulfanilamide	Total sulfa drug present	Concentrations Determined: Sulfanilamide	Total sulfa drug	Recovery: Sulfanilamide	Total sulfa drug
	Mg./cc.	*Mg./cc.*	*Mg./cc.*	*Mg./cc.*	*Mg./cc.*	%	%
1	0.05	0.0	0.05	0.053	0.053	106	106
2	0.025	0.025	0.05	0.028	0.046	111	92
3	0.025	0.0125	0.0375	0.024	0.041	96	109
4	0.05	0.0125	0.0625	0.047	0.069	94	110
5	0.0125	0.025	0.0375	0.013	0.032	104	86

Mode of Analysis

The apparatus used for the analysis is shown in Figure 3. It consists of a source, *A*, rich in lines between 310 and 260 mμ, and a glass indicator (014) which fluoresces when absorbing light of this wave length. The sample is placed in one side of the cell, *C*, and the concentration of sulfanilamide in the other side is varied until the two fluorescent fields show the same intensity. By interchanging the filters, *F*, we can differentiate the free sulfanilamide from the acetylated form.

The principles employed in executing the analysis can be understood most clearly by reference to Figure 2, which contains the transmission spectrum of each component of the instrument.

Spectra 5 and 3 are those of sulfanilamide and the acetyl derivative. Spectrum 6 is that of Corning 014 glass which fluoresces in the blue under the influence of the light it absorbs. The absorption range of the latter is almost identical with that of free sulfanilamide. It can be used, consequently, as a detector to determine the amount of light absorbed by the drug. A piece of Corning 9860 glass was placed before the arc in order to cut out the visible spectrum, and its absorption spectrum is shown as No. 7. When this is used with a 0.3-cm. (0.125-inch) cell of benzene we get spectrum 4. This combination, F_1, absorbs all the light below 280 mμ. Spectrum 2 is the transmission of Corning glass 9840. This filter in combination with Corning 9860 absorbs the light between 320 and 285 mμ and has the transmission of 9860 below 285 mμ. The set is employed as filter F_2.

In performing the analysis, a shield was used to shut off from view all but the lower part of the fluorescing field, *J*. The sample to be analyzed was placed in one side, *M*, of cell *C*, and 2 cc. of water were introduced into the other side, *N*. (The partition between the two sides of the cell was very thin, so that the two halves of the field, *J*, were nearly conterminous.) With filter F_1 at *F* the fluorescent fields received light of wave lengths that would be absorbed only by free sulfanilamide. Next, measured quantities of free sulfanilamide solution were added to *N* until fields *J* had the same visual intensity. The concentration of free sulfanilamide was then the same in both branches of the cell and could be calculated.

By substituting F_2 for F_1, only the light that was absorbed by both types of the drug was transmitted; hence if the sample contained any acetylated sulfanilamide, the fluorescing field in side *M* would be weaker than the one in *N*. It was assumed that both the free and acetylated forms had the same extinction in the spectral range transmitted, and more of the free sulfanilamide solution was added to *N* until balance was again achieved. Within the limits of this assumption the total sulfa drug concentration in side *M* was then equal to the free sulfanilamide concentration in *N* and was calculated. By subtraction the amounts of free and acetylated sulfanilamide were both known.

Aqueous solutions containing free and acetylated sulfanilamide were analyzed in this manner. An instrument was improvised from available equipment, employing the crude optical design of Figure 2 and a cell capacity of 3.5 ml. in each side. With mixtures of free sulfanilamide and acetylated sulfanilamide in which the total sulfa drug concentration was of the order of 0.05 mg. per cc., an accuracy of ±10 per cent was obtained in determining the amount of free sulfanilamide (filter F_1 at *F*), and of ±15 per cent in estimating the total sulfa drug content (filter F_2 at *F*). One set of data is shown in Table I. The accuracy could be increased appreciably, however, by improving the optical design and adjusting the cell size. The accuracy was limited largely by the exactitude with which the solution volumes could be measured. The total time required for analysis varied between 3 and 5 minutes.

In order to extend the method to the determination of these drugs in the blood, it will be necessary to observe certain precautions.

When drawing the blood and precipitating the protein, it is essential to prevent the introduction of compounds whose absorption spectra overlap the region 320 to 260 mμ. To illustrate, sodium oxalate would be unsatisfactory for preventing coagulation of the blood, but sodium citrate would be ideal for this use, since its ultraviolet absorption begins at wave lengths shorter than 260 mμ (the cutoff point of Corning 9860 glass). Dilute trichloroacetic acid could be used to precipitate the protein, although some other method might be more satisfactory.

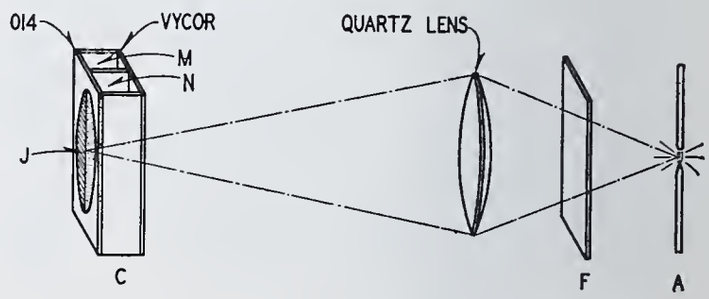

FIGURE 3. DIAGRAM OF APPARATUS

When these precautions are followed, it may be possible to carry out the analysis of the blood (after the protein is precipitated) in the manner described above for aqueous solutions. This phase has not been investigated by the author, but the procedure is being suggested as the basis of a method for the rapid determination of these substances in the blood.

Acknowledgment

The author is indebted to Dr. Schinn and Dr. Cooper of the Western Pennsylvania Hospital of Pittsburgh for supplying the compounds used as well as for their helpful suggestions and stimulating criticisms.

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Determination of Active Oxygen in the Presence of Barium and Lead

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DURING the past two years, the Chemical Laboratory of the Geological Survey has made a number of complete analyses of manganese minerals, in connection with studies of ores of manganese, a strategic metal. One of the determinations required is the percentage of "active" or "available" oxygen. This is usually easily made by adding to a weighed sample an excess of standard oxalic acid or sodium oxalate solution plus a little sulfuric acid, heating until solution of the mineral is complete, and back-titrating the excess oxalate with potassium permanganate.

This method, however, is inapplicable to the analysis of the barium manganate minerals psilomelane and hollandite and the lead manganate mineral coronadite. Psilomelane is of very common occurrence, and the other two minerals are occasionally encountered. When these minerals are heated with oxalic and sulfuric acids, the precipitated barium or lead sulfate coats undissolved particles of the mineral and prevents them from reacting, with consequent low results for active oxygen. For the same reason, this method cannot be used for the determination of active oxygen in lead dioxide (PbO_2), in red lead (Pb_3O_4), or in barium or strontium peroxides.

Several methods have been proposed for this determination. Four of these were studied in 1937 by Mrgudich and Clark (*1*), whose results may be summarized briefly:

1. The Lux method, involving solution in oxalic acid and nitric acid and the back-titration with permanganate, was found to be inaccurate because of a side reaction between nitric acid and oxalic acid.
2. The Schaeffer method, involving solution in hydrogen peroxide and nitric acid and back-titration with permanganate, was found to be inaccurate.
3. The modified Bunsen method, involving treatment with hot hydrochloric acid, absorption of the evolved chlorine in potassium iodide solution, and titration of the liberated iodine with thiosulfate, was found to be slow and cumbersome and to require special precautions to obtain complete absorption of chlorine.
4. The modified Diehl-Topf method, involving solution in acetic acid buffered with sodium acetate in the presence of excess potassium iodide, and titration of the liberated iodine with thiosulfate, was found to be accurate and fairly rapid.

The first three methods were accordingly discarded from consideration. The Diehl-Topf method, though apparently excellent for red lead, is unsuitable for the determination of active oxygen in manganese minerals, because these usually contain ferric iron which reacts with potassium iodide, liberating iodine. Not only do many manganese ores contain admixed ferric oxides, but the manganese minerals themselves often contain appreciable amounts (up to 11 per cent) of ferric oxide.

Mrgudich and Clark suggested a fifth method, involving solution in oxalic acid plus concentrated perchloric acid (50 per cent), dilution, and back-titration electrometrically with ceric sulfate. They obtained reproducible and apparently accurate results in this way. It seemed worth while, however, to attempt to modify their method, particularly with respect to two features: (1) The amount of acid used by Mrgudich and Clark (25 ml. of 72 per cent perchloric acid) seemed to be excessive; (2) electrometric titration with ceric sulfate happened to be inconvenient.

As shown below, it has been found that the amount of perchloric acid used may be diminished to less than one third the amount recommended by Mrgudich and Clark, and that the titration may be made with permanganate. There is no loss in accuracy or in speed, and no special technique is required.

TABLE I. BLANK RUNS

No.	0.06035 *N* Oxalic Acid *Ml.*	H_2SO_4 Concn. *Vol. %*	Treatment	Time *Min.*	0.0797 *N* $KMnO_4$ *Ml.*
15	50	5	80°	0[a]	37.84
18	50	5	Boiled	45	37.85
13	50	20	Boiled	45	37.82
25	50	30	80°	0[a]	37.88
22	50	30	Boiled	45	36.92
4	50	30	Boiled	45	36.76
		$HClO_4$ Concn.			
11	50	5	Boiled	45	37.83
9	50	20	Boiled	45	37.86
12	50	25	Boiled	45	37.80
21	50	30	80°	0[a]	37.87
14	50	30	Boiled	45	37.82
17	50	30	Boiled	45	37.48
26	50	50	Steam bath	60	37.83
27	50	50	Steam bath	45	37.84

[a] Heated to 80° and immediately titrated.

Experimental

A series of blanks was run with 50 ml. of 0.06035 *N* oxalic acid and varying amounts of sulfuric and perchloric acid in order to determine whether any side reaction occurred which consumed oxalic acid. Typical results, given in Table I, show that in sulfuric acid solution of moderate concentration there is no side reaction, but that some reaction which consumes oxalic acid occurs when 30 per cent sulfuric acid is boiled with oxalic acid for 45 minutes. This suggests the precaution of not allowing solutions to concentrate too far while the sample is being dissolved.

In perchloric acid solution, no appreciable side reaction occurred in acid of moderate concentration. When 30 per cent solutions of perchloric acid were boiled with oxalic acid, the titer was usually unaffected, but sometimes, as indicated, it was appreciably lower than the normal titer, as though some side reaction had occurred. It will be noted that 50 per cent solutions, heated at about 95° in the steam bath, showed normal titer. This is in agreement with the results of Mrgudich and Clark. The small amount of side reaction which perhaps occurred in 30 per cent acid may be due to the higher temperature during the heating, about 110° compared to 95° for the 50 per cent solution.

Table II shows some analyses by different procedures and the time required for complete solution. It is evident that neither speed nor accuracy is lost by the modifications in procedure suggested here; in fact, the 50 per cent acid seemed to require a little longer for complete solution of the mineral, perhaps because the solutions heated in the steam bath were relatively quiescent and less well mixed than the boiling solutions of lower acid concentration.

Mrgudich and Clark made comparative analyses of the same lead dioxide sample by different methods, but, as the purity of lead dioxide cannot be determined, they presented no data to show that the values obtained are the true figures.

Table II. Analyses

Material		Acid Used	Time for Solution	Active Oxygen
Gram		%	*Min.*	%
PbO_2	0.3300	$HClO_4$ 5	55	6.18
PbO_2	0.3300	$HClO_4$ 5	50	6.16
PbO_2	0.3300	$HClO_4$ 30	48	6.17
PbO_2	0.3300	$HClO_4$ 50	65	6.05
Pb_3O_4	0.7000	$HClO_4$ 5	12	2.06
Pb_3O_4	0.7000	$HClO_4$ 30	10	2.05
Pb_3O_4	0.7000	$HClO_4$ 50	12	2.07
Cryptomelane (*2*)	0.1100	$HClO_4$ 5	30	15.31
Cryptomelane (*2*)	0.1100	$HClO_4$ 5	Overnight	15.26
Cryptomelane (*2*)	0.1100	H_2SO_4 5	25	15.29
Cryptomelane (*2*)	0.1100	$HClO_4$ 50	40	15.33

Table III. Duplicate Analyses

	Active Oxygen 5% H_2SO_4	5% $HClO_4$
	%	%
Cryptomelane (*2*)		
Urucum, Brazil	14.52	14.56
Philipsburg, Mont.	16.07	15.99
Deming, N. Mex.	15.92	15.94
Sugar Stick, Ark.	14.97	15.12
Pyrolusite, Lake Valley, N. Mex.	17.89	17.86
Pyrolusite, Cuba	16.32	16.30
Rancieite, Cuba	13.80	13.82

The last of the three samples in Table II contained no lead and only 0.13 per cent barium oxide, so that the determination could be made using sulfuric acid. The results with sulfuric acid and perchloric acid are in excellent agreement. A number of similar samples have been run in duplicate, using sulfuric acid for one and perchloric acid for the other, with the results shown in Table III. It is evident that the perchloric acid method gives correct answers for these samples and it is to be presumed that the values obtained on material containing lead or barium are also correct.

It seemed probable that phosphoric acid could also be substituted for sulfuric acid in this determination. Experiments showed, however, that the c. p. phosphoric acid available gave fading and rather uncertain end points, presumably caused by the presence of some impurity. A few determinations made with 5 per cent phosphoric acid gave results in approximate agreement with those obtained using perchloric acid. No attempt was made to purify the phosphoric acid.

Procedure Recommended

To an appropriate weight of sample (0.15 gram of barium or lead manganate, 0.3 gram of lead dioxide or 0.7 gram of red lead), which is preferably ground to 200-mesh, add from a pipet, which need not be calibrated, 50 ml. of approximately 0.05 *N* oxalic acid solution, 8 ml. of 60 per cent perchloric acid, and 42 ml. of water. Place a short-stemmed funnel in the mouth of the flask to act as a condenser, and boil gently until solution is complete (usually about 20 minutes, but as long as 90 minutes may be required for resistant minerals such as hollandite). Add water from time to time if necessary to prevent the volume from diminishing to less than 50 ml. Dilute to 100 ml. and titrate directly at 80° with approximately 0.07 *N* potassium permanganate. The blank experiment to determine the permanganate equivalent of the oxalic acid needs only to be heated to 80° and need not be boiled as long as the actual determination, since there is no loss of titer.

Standard sodium oxalate solution or a weighed amount of sodium oxalate may be substituted for the oxalic acid solution, but is less convenient to use. Some samples may contain dark insoluble material. In such cases, the completeness of solution of the mineral containing active oxygen may be judged by noting whether bubbles of oxygen come off the undissolved particles. It is convenient at times to place the flask covered with a watch glass on the steam bath overnight. Solution is complete and the results obtained are accurate.

Summary

The method of Mrgudich and Clark is modified by substituting 5 per cent (by volume) perchloric acid for 50 per cent perchloric acid. Titration by potassium permanganate may be substituted for electrometric titration with ceric sulfate.

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New Reagents for Sodium

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SODIUM reagents consisting of uranyl acetate and certain divalent metal acetates in dilute acetic acid solution have come into extensive use for the detection and determination of sodium. Unfortunately, all such reagents now in use are more or less sensitive toward lithium, so that they are not satisfactory for the detection or determination of sodium in the presence of appreciable quantities of lithium. An indication was found, however, in an investigation by Caley and Baker (*1*) that a sodium reagent containing uranyl acetate and cupric acetate is less sensitive toward lithium than other reagents of this general type. The value of such a reagent for the detection and determination of sodium in the presence of lithium was the principal subject of the present investigation.

Aqueous Cupric Acetate–Uranyl Acetate Reagent

By means of tests with a series of trial reagents in which the concentrations of the components were systematically varied, it was found that a reagent of the following composition gave the most satisfactory results:

Uranyl acetate dihydrate	88 grams
Cupric acetate monohydrate	88 grams
Glacial acetic acid	60 ml.
Water	To 1000 ml.

The salts are dissolved in the acetic acid and nearly all the necessary water at a temperature of 50° to 60° C., after which the solution is cooled to room temperature, adjusted to final volume with water, and allowed to stand a day. The solution is then maintained at 20° C. while being stirred vigorously for about 2 hours with a mechanical stirrer, and is next filtered through a dry filter to remove the small amounts of precipitated salts. The reagent prepared in this way is stable.

The sensitivity of this reagent toward sodium and lithium is indicated in Table I. Though it is evidently insensitive toward lithium, it is unfortunately also not very sensitive toward sodium. Experiments with the trial reagents showed that the sensitivity toward lithium can be further reduced by decreasing the concentration of uranyl acetate in the reagent, but unfortunately this is paralleled by a decrease in sensitivity toward sodium. An aqueous reagent of this type is obviously not satisfactory for the accurate quantitative determination of sodium, however useful it may be for the qualitative detection of sodium in the presence of considerable lithium.

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Table I. Sensitivity of Aqueous Cupric Acetate–Uranyl Acetate Reagent toward Sodium and Lithium

Ion	Amount Present	Reaction on Addition of Stated Volume of Reagent to 1 Ml. of Chloride Solution			
	Mg.	*1 ml.*	*3 ml.*	*5 ml.*	*10 ml.*
Na	2	+	+	+	+
	1	−	+	+	+
	0.5	−	+	+	+
	0.3	−	−	−	−
Li	20	+	+	+	+
	10	−	−	−	−

Alcoholic Cupric Acetate–Uranyl Acetate Reagent

By replacing part of the water in the aqueous reagent with ethyl alcohol and altering the proportion of the other components, it was found through systematic experiments that a reagent could be prepared that was at the same time more insensitive toward lithium and more sensitive toward sodium than the aqueous reagent. The composition of this alcoholic reagent is as follows:

Uranyl acetate dihydrate	40 grams
Cupric acetate monohydrate	25 grams
Glacial acetic acid	100 ml.
Ethyl alcohol, 95%	500 ml.
Water	450 ml.

The salts are dissolved in the water and acetic acid at a temperature of 50° to 60° C., and then after cooling down to room temperature the alcohol is added with constant stirring. After standing at least one day, preferably 2 or 3, the mixture is stirred and filtered in the same way as described for the preparation of the aqueous reagent. The longer time of standing in the preparation of the alcoholic reagent is recommended because the excess of salts separates more slowly than in the preparation of the aqueous reagent. This alcoholic reagent is somewhat less stable than the aqueous, particularly in the presence of light. However, a specimen of this reagent which had been protected from strong light was still effective a year after its preparation.

In Table II is indicated the sensitivity of this reagent toward sodium and lithium. As little as 0.1 mg. of sodium may be detected if sufficient volume of reagent is used, and as much as 50 mg. of lithium will not produce a precipitate when the volume of reagent amounts to 20 ml. With this same volume of reagent as much as 40 mg. of potassium present as chloride will also not produce a precipitate. When, however, potassium is present as sulfate less potassium will result in the formation of a precipitate, in part because of the low solubility of potassium sulfate in strong alcoholic solution. This and similar interference from the low solubility of a salt in alcoholic solution may be obviated by adding alcohol to the test solution until the concentration is about 50 per cent, filtering off any precipitate, and then adding the reagent. When the test is performed in this fashion 0.1 mg. of sodium present in the original test solution may still be detected. Ammonium alone even in high concentration does not produce a precipitate with this reagent. The effect on the sensitivity of the reagent toward sodium when other alkali ions are also present is indicated in the tables and discussion on the quantitative application of this reagent.

Anions such as ferrocyanide or phosphate which precipitate copper or uranium interfere with the detection of sodium with this reagent. The presence of much free strong acid appreciably reduces the sensitivity of this reagent toward sodium—for example, even 1 mg. of sodium produces no precipitate when the test solution is 6 *N* in hydrochloric acid.

This reagent is stable for as long as a year if kept out of strong light. It rapidly deteriorates if exposed to sunlight because of photochemical reduction of the uranyl salt. Because of this the reagent should always be kept in a dark bottle.

Composition of Sodium and Lithium Precipitates

By analogy with the triple acetates produced by other sodium reagents, it is to be expected that those produced by this aqueous cupric acetate–uranyl acetate reagent would have the composition

$$3UO_2(C_2H_3O_2)_2.Cu(C_2H_3O_2)_2.MC_2H_3O_2.XH_2O$$

where M is sodium or lithium and X approximately 6.

Samples of the sodium salt were prepared by precipitating 20 mg. of sodium contained in 2 ml. of solution with 20 ml. of the aqueous reagent, filtering the precipitated salt on a glass crucible, washing it sparingly with 95 per cent ethyl alcohol, and drying in air to constant weight. In order to prepare sufficient lithium salt for analysis, it was necessary to have 250 mg. of lithium present in 5 ml. of solution, and to add 25 ml. of reagent. The lithium salt was washed and dried in the same way.

The composition of the sodium triple salt was checked by copper and uranium determinations. Samples were dissolved in dilute sulfuric acid, and, after being evaporated to fumes of sulfur trioxide and diluted, the solutions of the samples were electrolyzed for the determination of copper. The uranium in the filtrates from the electrolyses was precipitated as ammonium diuranate by means of carbonate-free ammonium hydroxide and weighed as U_3O_8. The analyses of the lithium triple salt included determinations of the lithium content, lithium being determined in the filtrates from the uranium separations by evaporating with dilute sulfuric acid in a platinum dish and weighing the residue as Li_2SO_4. Because of the method of analysis it is likely that these lithium determinations were a little high.

Table II. Sensitivity of Alcoholic Cupric Acetate–Uranyl Acetate Reagent toward Sodium and Lithium

Ion	Amount Present	Reaction on Addition of Stated Volume of Reagent to 1 Ml. of Chloride Solution				
	Mg.	*1 ml.*	*3 ml.*	*5 ml.*	*10 ml.*	*20 ml.*
Na	0.3	+	+	+	+	+
	0.2	−	+	+	+	+
	0.1	−	−	+	+	+
Li	50	+	+	+	+	−
	25	−	−	−	−	−

Table III. Analyses of Precipitates

(Produced by adding an aqueous cupric acetate–uranyl acetate reagent to solutions containing sodium or lithium)

		Copper %	Uranium %
	Sodium Precipitate		
Found		4.15	46.50
		4.16	46.50
Calculated for a hexahydrate		4.14	46.50
	Lithium %		
	Lithium Precipitate		
Found	0.55	4.21	46.90
	0.54	4.22	46.93
Calculated for a hexahydrate	0.45	4.16	46.79

The results of the analyses are shown in Table III. It is evident that both the sodium and the lithium salts precipitated by the aqueous cupric acetate–uranyl acetate reagent are of the same type as those precipitated by the other aqueous sodium reagents.

Although the precipitate produced by adding the alcoholic reagent to sodium solutions was found to be a triple salt of the same type as regards the ratios of component salts, this triple salt was, surprisingly enough, solvated with both water and alcohol. That alcohol is actually a chemically combined component of the salt was shown by the fact that it is retained in constant proportion in preparations dried at different elevated temperatures, and is retained even at temperatures above the boiling point of the alcohol. No sensible loss in weight

Table IV. Analysis of Precipitate

(Produced by adding an alcoholic cupric acetate–uranyl acetate reagent to solutions containing sodium)

	Copper	Uranium	Alcohol
	%	%	%
Found	4.00	45.15	4.04
	4.02	45.22	4.07
	4.04	45.14	...
Calculated for Possible Solvated Salts			
I	4.02	45.13	2.91
II	3.98	44.74	4.33
III	4.00	44.99	4.35
IV	4.03	45.25	4.38
V	3.99	44.85	5.78

Key to Possible Solvated Salts

I. Triple salt solvated with 6 H_2O and 1 C_2H_5OH, M. W. = 1582.4
II. Triple salt solvated with $5^1/_2$ H_2O and $1^1/_2$ C_2H_5OH, M. W. = 1596.4
III. Triple salt solvated with 5 H_2O and $1^1/_2$ C_2H_5OH, M. W. = 1587.4
IV. Triple salt solvated with $4^1/_2$ H_2O and $1^1/_2$ C_2H_5OH, M. W. = 1578.4
V. Triple salt solvated with 4 H_2O and 2 C_2H_5OH, M. W. = 1592.4

occurred on heating to 60° C. a preparation which had been dried to constant weight in air at room temperature. On heating this up to 85° C. a slight loss in weight occurred which amounted to 0.5 per cent when constancy was reached. This was only a small fraction of the alcohol content of the salt, and it is not certain whether this loss in weight was due entirely to escape of alcohol or to escape of both water and alcohol. On heating this same preparation to 110° C. a gradual loss in weight occurred extending over a period of 10 weeks. This loss in weight was accompanied by a general decomposition of the salt, as was shown by a color change from a tan to a deep green.

Samples of the sodium salt for analysis were prepared in a manner analogous to that employed in preparing the samples of sodium salt precipitated by the aqueous reagent, and the copper and uranium determinations were made in the same way. The determinations of the alcohol content were made on separate samples by oxidizing them for 1 hour at 100° C. with a measured amount of standard potassium bichromate acidulated with sulfuric acid, the excess of bichromate being determined on cooling by potentiometric titration with a freshly standardized solution of ferrous sulfate. It was not found possible to estimate the alcohol content by measuring the loss in weight on heating, since at a temperature at which the alcohol was driven off some water was also driven off. Nor was it found possible to estimate by the same means the total content of alcohol and water, since at the temperature required to drive both these off decomposition of the component acetates began. However, the total degree of solvation could be estimated from a rough determination of the molecular weight of the salt, this being done by precipitating quantitatively known weights of sodium and weighing the resulting precipitates. It was found that the molecular weight must lie between 1575 and 1600.

Results of the analyses and a tabulation of the composition of the various possible solvates are given in Table IV. This triple salt is solvated with about 1.5 molecules of alcohol and with 4.5 or 5 molecules of water. Subsequent to the work reported in this paper, determinations of the water content of this triple salt by means of the Karl Fischer reagent have indicated that the water content is close to 5 molecules (*4*). Thus the composition of the salt is reasonably well established.

If the assumption had been made that the salt was solvated with water only, and the degree of solvation had been estimated from the copper and uranium determinations, it would have been concluded that this salt was an octahydrate. This is interesting, inasmuch as Kahane (*3*) reported that sodium magnesium uranyl acetate precipitated by an alcoholic magnesium acetate–uranyl acetate reagent is an octahydrate. An analysis of samples of sodium magnesium uranyl acetate precipitated by Kahane's reagent showed that this salt is not an octahydrate as reported by Kahane on the basis of determinations of the metal and acetate content but is a salt solvated with both water and alcohol. Two determinations of the alcohol content gave 4.17 and 4.18 per cent. The sodium salt precipitated by an alcoholic magnesium acetate–uranyl acetate reagent is thus analogous in composition to that precipitated by the alcoholic cupric acetate–uranyl acetate reagent. Confirmation of the presence of alcohol as an essential component in the precipitate produced by Kahane's reagent was found soon after this work was completed in an abstract of a paper by Schoorl (*5*). The presence of alcohol in this precipitate is a source of error that must be taken into account if sodium is to be determined by reduction of the uranium in a solution of the precipitate followed by titration with an oxidizing agent, as has been recommended by Kahane and others, since results are likely to be high because of the presence of alcohol as a second oxidizable substance.

Table V. Determinations of Sodium with Alcoholic Cupric Acetate–Uranyl Acetate Reagent

Reagent	Sodium Chloride Solution	Sodium Present	Sodium Found	Difference
Ml.	*Ml.*	*Mg.*	*Mg.*	*Mg.*
50	2	1.0	0.9	−0.1
100	5	1.0	1.0	±0.0
25	5	10.0	9.2	−0.8
50	5	10.0	10.0	±0.0
100	5	10.0	10.1	+0.1
125	5	10.0	9.8	−0.2
50	2	20.0	19.7	−0.3
50	2	20.0	20.0	±0.0
100	2	20.0	20.0	±0.0
100	5	20.0	20.0	±0.0
100	2	50.0	47.8	−2.2
150	5	50.0	48.4	−1.6
250	5	50.0	49.7	−0.3
350	2	50.0	49.9	−0.1

Quantitative Application of Alcoholic Reagent

The alcoholic cupric acetate–uranyl acetate reagent may be used for the quantitative determination of sodium with satisfactory results. In the trial determinations reported here the procedure used was essentially that of Caley and Foulk (*2*) employing the aqueous magnesium acetate–uranyl acetate reagent, except that the precipitate was collected on glass crucibles of medium porosity, and washing was performed with 95 per cent ethyl alcohol freshly saturated with sodium copper uranyl acetate. Moreover, the precipitate was dried at 60° C., not at 110° C. This was necessary because of its lower stability as compared to sodium magnesium uranyl acetate. The factor 0.0145 was used for converting the weight of dried precipitate into weight of sodium.

In Table V are shown results obtained with sodium present alone. It will be seen that by using a sufficient volume of reagent, amounts of sodium from 1 to 50 mg. may be determined with satisfactory accuracy. Experiments on the determination of quantities below 1 mg. indicated that 0.4 mg. is about the smallest quantity that may be successfully determined with this reagent when using ordinary volumes of reagent and sample solution.

In Table VI are shown results of determinations of sodium in the presence of lithium. Contrary to what was expected from the results of the qualitative experiments, the presence of lithium invariably caused a positive error in the sodium determinations. The magnitude of this error is apparently independent of the volume of reagent used, is decreased slightly by increasing the volume of sample solution, and is

Table VI. Determinations of Sodium in the Presence of Lithium

Reagent	Sample Solution	Lithium Present	Sodium Present	Sodium Found	Difference
Ml.	*Ml.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*
50	2	10.0	10.0	10.4	+0.4
		15.0	10.0	10.6	+0.6
		20.0	10.0	10.5	+0.5
		25.0	10.0	10.6	+0.6
100	2	10.0	10.0	10.5	+0.5
		15.0	10.0	10.8	+0.8
		20.0	10.0	10.6	+0.6
		25.0	10.0	10.7	+0.7
50	5	10.0	10.0	10.2	+0.2
		15.0	10.0	10.3	+0.3
		20.0	10.0	10.3	+0.3
		25.0	10.0	10.2	+0.2
		50.0	10.0	10.7	+0.7
100	5	10.0	10.0	10.3	+0.3
		15.0	10.0	10.6	+0.6
		20.0	10.0	10.4	+0.4
		25.0	10.0	10.6	+0.6
		50.0	10.0	11.1	+1.1
100	5	25.0	20.0	21.3	+1.3
		50.0	20.0	21.5	+1.5
		100.0	20.0	21.7	+1.7

Table VII. Determinations of Sodium in the Presence of Potassium

Reagent	Sample Solution	Potassium Present	Sodium Present	Sodium Found	Difference
Ml.	*Ml.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*
50	2	50	10.0	10.2	+ 0.2
50	5	50	10.0	10.0	± 0.0
50	5	100	10.0	10.1	+ 0.1
50	5	200	10.0	10.2	+ 0.2
50	5	300	10.0	15.5	+ 5.5
100	2	50	10.0	10.4	+ 0.4
100	5	50	10.0	10.1	+ 0.1
100	5	100	10.0	10.4	+ 0.4
100	5	200	10.0	10.8	+ 0.8
100	5	300	10.0	20.6	+10.6
100	5	50	20.0	19.5	− 0.5
100	5	100	20.0	20.4	+ 0.4
100	5	200	20.0	21.1	+ 1.1
100	5	300	20.0	22.8	+ 2.8

increased with increase in amount of sodium precipitated. As shown also by the results in Table VI, the error is by no means proportional to the amount of lithium present. The errors in these determinations of sodium in the presence of lithium with the cupric acetate–uranyl acetate reagent are much smaller than the errors which would result in similar determinations with the magnesium acetate–uranyl acetate or the zinc acetate–uranyl acetate reagents. These errors are not serious when quantities of lithium up to 10 mg. are present and when suitable volumes of reagent and sample solution are employed. It seems likely from the present results that determinations of sodium in the presence of larger amounts of lithium could be accomplished with satisfactory accuracy if a suitable small correction were applied to the final results. Thus for purposes where high accuracy is not required, the alcoholic cupric acetate–uranyl acetate reagent may be useful for estimating sodium in the presence of both small and large amounts of lithium. In Table VII are shown results of determinations of sodium in the presence of potassium with this reagent. Interference from potassium is not serious unless considerable amounts are present, the extent of the interference being in the same order of magnitude as that from other sodium reagents of this type. The effect of ammonium is illustrated by the results in Table VIII. In contrast to effects observed with other sodium reagents, slightly low results are obtained with the alcoholic cupric acetate–uranyl acetate reagent when considerable amounts of ammonium are present. However, the interference from ammonium is not serious from the standpoint of practical analysis because excessive amounts are so readily removed.

From a manipulative viewpoint the use of an alcoholic cupric acetate–uranyl acetate for quantitative work is slightly less convenient than the use of aqueous magnesium acetate–uranyl acetate or zinc acetate–uranyl acetate reagents, since the crystals of sodium copper uranyl acetate are more difficult to transfer than those of the triple acetates precipitated by these other reagents by reason of their greater coarseness, density, and tendency to adhere to the walls of the vessel in which the precipitation is made. In general, the alcoholic cupric acetate–uranyl acetate reagent offers no advantages over other sodium reagents of this type unless determinations of sodium are to be made in the presence of lithium.

Other Possible Reagents

An apparently heretofore unexplored possibility by which the triple salt method for sodium might be improved is the use of metal salts of certain substituted acetic acids and of certain homologs of acetic acid in the preparation of sodium reagents. Reagents prepared from uranium and magnesium chloroacetates, formates, propionates, and butyrates, modeled after the magnesium acetate–uranyl acetate reagent of Caley and Foulk (*2*), were studied. The magnesium-uranyl salt type was selected for study because of the ease by which the magnesium salts of the acids could be prepared. The magnesium chloroacetates were prepared by the dissolution of both pure magnesium and pure magnesium carbonate in the pure acids; the formates, propionates, and butyrates by the dissolution of pure magnesium. The uranyl salts were prepared by the dissolution of pure uranium trioxide in the acids.

Table VIII. Determinations of Sodium in the Presence of Ammonium

Reagent	Sample Solution	Ammonium Present	Sodium Present	Sodium Found	Difference
Ml.	*Ml.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*
50	2	100	10.0	9.9	−0.1
50	5	100	10.0	9.8	−0.2
50	5	200	10.0	9.9	−0.1
50	5	300	10.0	9.9	−0.1
50	5	400	10.0	9.7	−0.3
100	2	100	10.0	10.0	±0.0
100	5	100	10.0	9.8	−0.2
100	5	200	10.0	9.7	−0.3
100	5	300	10.0	9.7	−0.3
100	5	400	10.0	9.4	−0.6

These reagents were tested qualitatively for sensitivity toward sodium, lithium, and potassium by adding 10 ml. of reagent to 1 ml. of chloride solutions of these ions. Surprisingly enough, not one of those possible reagents was sensitive toward sodium. No precipitate was obtained with the chloroacetate and formate reagents, even when highly concentrated sodium chloride solutions were tested. The propionate reagent yielded only a slight precipitate with 50 mg. of sodium, and although the butyrate reagent gave a large initial precipitate with the same amount of sodium, this precipitate redissolved before all the reagent was added. With lithium solutions the chloroacetate and formate reagents yielded no precipitates. The propionate reagent was somewhat more sensitive toward lithium than toward sodium, since a large precipitate was obtained with 50 mg. and a slight precipitate with as little as 20 mg. The butyrate reagent failed to precipitate even highly concentrated lithium solutions. With potassium solutions the chloroacetate and formate reagents also yielded no precipitates. The propionate reagent yielded a moderate precipitate with 50 mg. of potassium but not with 20 mg. On the other hand, the butyrate re-

agent produced a very heavy precipitate with 50 mg., a heavy precipitate with 20 mg., and a slight precipitate with 5 mg. but no precipitate with 1 mg. In other words, the butyrate reagent turned out to be a selective but not very sensitive reagent for potassium.

Contrary to what might reasonably have been expected, these reagents closely analogous to the acetate type of reagent were found to be entirely ineffective for the detection or determination of sodium.

Acknowledgment

Much of the work reported in this paper was done by Lockhart B. Rogers while holding a J. T. Baker Chemical Company Fellowship in Analytical Chemistry at Princeton University for the year 1941–42.

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Determination of Alkalies in Glass

Denatured Alcohols as Substitutes for U. S. P. Grain Alcohol

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THE procedures (*3*) for determining sodium and potassium in soda-lime glasses specify the use of 95 per cent ethyl alcohol in the preparation of wash solutions. In the method for sodium, the precipitate of sodium zinc uranyl acetate is washed with a saturated solution of the triple acetate salt in 95 per cent ethyl alcohol. In the potassium procedure the potassium chloroplatinate precipitate is washed with 80 per cent ethyl alcohol.

Table I. Sodium Oxide Determinations on Glass

Alcohol Used	Na_2O, %
Alcohol denatured with 10% ether	16.81
	16.77
	16.81
Alcohol denatured with 10% acetone	16.79
	16.81
95% grain alcohol	16.80
	16.81
Bureau of Standards recommended value	16.83

The possibility of substituting one of the specially denatured alcohols for 95 per cent grain alcohol in the determination of sodium and potassium has been investigated. Two commercial denatured alcohols were selected for trial, one containing about 10 per cent of ethyl ether and the other about 10 per cent of acetone. These two products were compared with 95 per cent ethyl alcohol in a series of determinations of sodium and potassium in the National Bureau of Standards sample of soda-lime glass No. 128.

Sodium Determinations

Sodium (*3*) was precipitated as sodium zinc uranyl acetate and the precipitate was washed, first with the aqueous reagent, then with a solution of alcohol saturated with the triple acetate salt, and finally with ether. For purposes of comparison, quantities of the two selected denatured alcohols were saturated with the sodium salt and used in the same manner as the saturated wash solution prepared from 95 per cent grain alcohol.

Comparative results obtained on a series of 0.1-gram samples, Bureau of Standards glass No. 128, are given in Table I.

The percentages have been adjusted for the change in ignition loss on standard glass No. 128 occurring since the sample was distributed.

It is evident that the two denatured alcohols selected are satisfactory as substitutes for 95 per cent grain alcohol in the determination of sodium oxide in glass.

Potassium Determinations

Potassium (*3*) was determined by the modified Hicks method, in which the potassium is first separated as the chloroplatinate; this salt, after thorough washing with alcohol to eliminate soluble platinum compounds, is dissolved in hot water, then reduced to yield metallic platinum which can be separated and weighed.

The original Hicks procedure (*2*) specifies a wash solution of ethyl alcohol of at least 80 per cent strength. For purposes of comparison, each of the denatured alcohols in question was diluted with 10 per cent water (by volume), resulting in a concentration of ethyl alcohol, exclusive of the denaturant, of approximately 80 per cent. These solutions were compared with 80 per cent ethyl alcohol in a series of K_2O determinations on Bureau of Standards glass No. 128 (Table II).

The question of optimum concentration of alcohol for washing potassium chloroplatinate precipitates has been discussed very recently by Ford and Hughes (*1*). In a series of potassium determinations on a large number of fertilizer samples, they found that the chloroplatinate precipitate is slightly more soluble in 80 per cent than in 95 per cent ethyl alcohol. They conclude that undiluted 95 per cent alcohol is a little more satisfactory than the diluted form as a wash solution for potassium chloroplatinate.

The tests indicate that the two denatured alcohols in question may be used satisfactorily to replace 95 per cent grain alcohol in the potassium procedure.

Table II. Potassium Oxide Determinations on Glass

Wash Solution	K_2O, %
90 parts of ether-denatured alcohol + 10 parts of water	0.98
	0.99
90 parts of acetone-denatured alcohol + 10 parts of water	0.95
	0.97
80 parts of 95% alcohol + 20 parts of water	0.99
	0.97
Bureau of Standards recommended value	0.99

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Spray Residues of Tartar Emetic on Citrus Leaves

Determination by Iodometric Titration

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TARTAR emetic is now used to control certain species of thrips, especially the citrus thrips, *Scirtothrips citri* (Moult.), on lemons and oranges. When it is used for the control of the citrus thrips, it is combined with sucrose and applied with a broom gun or a high-speed blower sprayer. During the 1940 season in California a spray solution was used containing 1.5 pounds (680 grams) of tartar emetic, 2 pounds (900 grams) of sucrose, and 100 gallons (378 liters) of water. Davidson, Pulley, and Cassil (*1*) have proposed a modified Gutzeit method for the determination of tartar emetic spray residues. However, it was desired to develop a simpler method that would eliminate ashing of the leaf material.

Table I. Tartar Emetic Deposits on Orange Foliage

(Sprayed with 1.5 pounds of tartar emetic and 2 pounds of sugar in 100 gallons)

Time from Spraying to Sampling	No. of Whole Leaves Taken	Deposit[a]
Days		*Micrograms/sq. cm.*
	Plot 1 at Redlands	
0	50	9.4
	50	7.8
	48	10.3
	50	6.6
	49	10.7
	50	7.0
Rain		
13	50	2.6
	50	3.0
	50	1.9
	51	1.5
21	25	1.9
	25	1.3
	27	1.5
	24	1.2
	Plot 2 at Redlands	
7	25	4.5
	25	5.1
	25	6.7
	25	6.4
	Plot 1 at San Bernardino	
	49	9.2
	50	7.4
Rain		
13	47	1.5
	50	1.1

[a] A correction factor, 1.80 micrograms per sq. cm. for leaf material.

Direct titration with iodine solution in the presence of sodium bicarbonate is a recognized means of determining antimony in solution in the trivalent form in which it exists in tartar emetic [$K(SbO)C_4H_4O_6 . {}^1/_2H_2O$]. Preliminary experiments showed that this titration was not significantly altered by the presence of sucrose, or by tartaric acid, which it was proposed to use as an aid to the removal of the spray from the leaves. Additional experiments showed that known amounts of tartar emetic applied to leaves could be quantitatively recovered by immediate washing with 1 per cent tartaric acid solution. If the treated leaves were allowed to dry overnight, or were kept in a moist chamber for several days, the titer was greater than the tartar emetic applied, but when corrected for the leaf material the final recoveries averaged about 98 per cent.

For example, 3.07 ml. of solution containing 5.78 mg. of tartar emetic were added to the surface of 13 leaves and allowed to dry overnight. The following morning the residue was removed with tartaric acid and titrated with iodine; 5.84 mg. of tartar emetic were removed from the leaves compared with 5.78 mg. added. The correction factor for the leaves used was 0.02 mg. per 32.6 sq. cm. of leaf surface (average area of leaf). When the correction factor was applied for the leaf material (0.26) the actual amount removed was 5.58 mg. compared with 5.78 mg. added. In another test the leaves were allowed to stand for 3 days in a standard moisture cage (*2*). When they were removed, washed with tartaric acid, and titrated with iodine, 8.82 mg. of tartar emetic were found compared with 8.51 mg. added. When the above figure was corrected (0.02 × 17 leaves) for the leaf material, 8.82 mg. − 0.34 mg. = 8.48 mg.

The use of the method is not valid in the presence of arsenites, but these compounds are seldom, if ever, applied to citrus trees.

Procedure

Replicate samples are collected from a plot of trees sprayed with tartar emetic by selecting at random a few leaves from each of several trees until approximately 25 or 50 have been taken. The replicate samples need not come from the same trees. Similar samples are collected from unsprayed trees. The leaf material is placed immediately in a portable ice box and taken to the laboratory, where it is stored in a refrigerator until analyzed. (Samples have been satisfactorily kept for 18 days.) As soon as practicable the leaves are washed with water containing 1 per cent of tartaric acid, first by agitation in a container and then individually by means of a wash bottle. The washings are made to 250 ml. for a 25-leaf sample and to 500 ml. for one of 50 leaves.

Aliquants of 100 ml. are neutralized with solid sodium bicarbonate and enough is added to saturate the solution. It is immediately titrated with standard approximately 0.01 *N* iodine solution, using freshly prepared starch solution as indicator. The iodine must be added at a uniform rate (approximately 10 seconds per ml.).

After the leaves have been washed, their outlines are traced on paper and the area is determined with a planimeter. The results in this paper are reported in terms of residue per square centimeter of leaf, and not on the basis of total leaf surface.

The correction to be applied is determined by carrying the unsprayed samples through the same procedure.

For residues on lemons approximately 15 fruits are taken as a sample. They are assumed to be prolate spheroids, and the area is calculated from measurements of the major and minor axes.

That the titration obtained is really due to antimony is confirmed by Reinsch's test, in which a strip of bright copper is boiled for 5 minutes in a portion of the solution to which has been added one sixth its volume of concentrated hydrochloric acid. A black or violet lustrous deposit is obtained.

Table II. Tartar Emetic Deposits on Lemon Foliage and Lemons

(At San Fernando, Calif., 12 days after spraying with 1.5 pounds of tartar emetic and 2 pounds of sugar in 100 gallons)

Sprayed Material Analyzed	No.	Deposit
		Micrograms/sq. cm.
Leaves[a]	51	1.6
	49	2.4
Lemons[a]	14	0.9
	14	0.7

[a] A correction factor of 1.50 micrograms per sq. cm. was used for leaves and 1.60 micrograms per sq. cm. for lemons.

Results

With this technique tartar emetic deposits have been determined on citrus foliage and on lemons from groves in several sections of southern California. The results are shown in Tables I and II.

Summary

The antimony residues from spraying citrus trees with tartar emetic and sugar can be removed from the leaves by washing with dilute tartaric acid solution and determined by titrating with standard iodine solution. The residues from orange foliage from a spray containing 1.5 pounds of tartar emetic and 2 pounds of sugar per 100 gallons were found to contain antimony equivalent to 6.6 to 10.7 micrograms of tartar emetic per square centimeter immediately after spraying, but these residues were greatly reduced by rain.

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Determining an Alkali Carbonate in the Presence of an Alkali Bicarbonate

A Colorimetric Method

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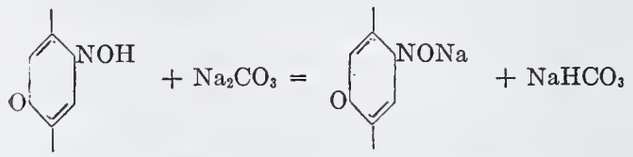
THE carbonates and bicarbonates of the alkali metals are important industrial chemicals and useful chemical reagents, since they are the salts of a strong base and a weak acid and are the only readily available salts of these anions which are soluble in water.

It is frequently necessary to distinguish between the carbonate and bicarbonate of an alkali metal, and to determine one in the presence of the other, since solutions of bicarbonates lose carbon dioxide to revert to the corresponding carbonate and solutions of the carbonates absorb carbon dioxide to become contaminated with the bicarbonate.

The pH of an alkali carbonate solution is higher than that of the corresponding bicarbonate; hence, these anions may be distinguished by their behavior with an indicator such as turmeric (*7*), which is reddened by solutions of an alkali carbonate but not by solutions of an alkali bicarbonate. Mercuric chloride (*6*), which gives a brownish-red precipitate with solutions of an alkali carbonate and a white precipitate with solutions of the bicarbonate, has been used to distinguish between these anions; as has magnesium sulfate (*5*), which precipitates at room temperature with alkali carbonates but not with the corresponding bicarbonates.

Qualitative Procedure

In a study of the tautomerism of *p*-nitrosothymol and thymoquinone monoxime (*9*) it was observed that a solution of sodium carbonate was alkaline enough to tautomerize the colorless *p*-nitrosothymol into thymoquinone monoxime with the simultaneous production of a red color due to the presence of the anion of the sodium salt of the oxime:



A solution of sodium bicarbonate under the same conditions produces no color or a very faint yellow color, depending on the amount of carbonate contamination in the bicarbonate sample. Thus *p*-nitrosothymol can be used to distinguish between alkali carbonates and bicarbonates and provides a qualitative test for the presence of an alkali carbonate in a sample of an alkali bicarbonate.

p-Nitrosothymol is available from the Eastman Kodak Company, Rochester, N. Y. It can also be prepared in almost quantitative yields by the method of Kremers and Wakeman (*3*). Purification of *p*-nitrosothymol can be accomplished by recrystallization from benzene or from diluted alcohol with the use of activated carbon.

Quantitative Procedure

There are standard procedures (*10*) for titrating an alkali carbonate in the presence of an alkali bicarbonate by the use of selected indicators. The accuracy of these methods depends upon several factors, especially the choice of the indicator, but under no condition is it exact when the amount of bicarbonate is proportionately large (*2*).

For determining inadmissible amounts of carbonate in official samples of the alkali bicarbonates, the U. S. Pharmacopoeia XI (*11*) requires that a 1-gram sample of the salt be not alkaline to phenolphthalein after it has been dissolved in 20 ml. of distilled water below 15° C. and treated with 2 ml. of 0.1 *N* hydrochloric acid. The British Pharmacopoeial method (*1*) is similar, except that thymol blue is used as the indicator. While these pharmacopeial methods serve the purpose for which they are intended, they are not quantitative.

The qualitative test using *p*-nitrosothymol was investigated to determine whether the intensity of the color produced with the indicator was in direct ratio to the amount of alkali carbonate present, so that it could be used for quantitatively determining an alkali carbonate in the presence of the corresponding bicarbonate.

Experimental

To a series of Nessler tubes, arranged in a rack fitted with a white porcelain base, were added equal volumes of sodium carbonate solutions of graduated molarity. To each tube was then added approximately twice the calculated quantity of *p*-nitrosothymol previously dissolved in enough neutral acetone or neutral dioxane to give a 0.35 *M* solution. (The *p*-nitrosothymol was dissolved in the solvent to facilitate its admixture with the carbonate solutions.) The tubes were shaken for from 10 to 15 minutes, and the excess *p*-nitrosothymol was filtered off. These filtrates provide the reference standards. An equal volume of a solution containing an unknown amount of alkali carbonate was treated in like manner, after which its intensity of color was compared to those of the reference standards for the determination of its alkali carbonate content.

By this procedure, using a blank composed of *p*-nitrosothymol and distilled water, it was found that the color produced by the indicator in the presence of 0.0001 *M* solutions of sodium carbonate could be detected with the unaided eye. From this molarity the method is applicable in concentrations up to 0.1 *M* sodium carbonate, above which the color is too

intense for comparisons. Near the lower concentration it is possible to distinguish between a 0.0001 and a 0.00015 *M* sodium carbonate solution, and at the higher concentration between a 0.1 and 0.09 *M* solution.

Three different procedures were used to check the accuracy of the *p*-nitrosothymol method against other methods used to determine alkali carbonates in the presence of alkali bicarbonates.

A solution of sodium carbonate of known molarity was prepared by dissolving reagent monohydrated sodium carbonate in distilled water. Measured portions of this solution were assayed by titration with standardized hydrochloric acid, using methyl orange as an indicator. Identical samples were assayed by the use of *p*-nitrosothymol in the manner described above. Twelve color reference standards ranging from 0.01075 to 0.01625 *M* with respect to sodium carbonate were used, and each successive tube in the series differed to the extent of 0.0005 in molarity. The results by the two methods are:

Molarity of solution with respect to Na_2CO_3	
True molarity	0.01325
As determined with HCl (average of 3 determinations)	0.01338
As determined with *p*-nitrosothymol (average of 5 readings)	0.01340

A 0.15 *M* solution of sodium bicarbonate was treated with carbon dioxide until it no longer gave a color with *p*-nitrosothymol. To this solution were added enough sodium carbonate and distilled water to give a solution which was 0.075 *M* with respect to sodium bicarbonate and 0.00525 *M* with respect to sodium carbonate. The amount of sodium carbonate in this solution was then determined by Kuster's method (*4*), which consists of titrating the solution at 0° C. in the presence of an alkali chloride with standardized acid, using phenolphthalein as the indicator. The amount of sodium carbonate was determined also with the use of *p*-nitrosothymol in the manner described above. Twelve color reference standards which ranged from 0.00275 to 0.00825 *M* with respect to sodium carbonate were used, and each successive tube in the series differed to the extent of 0.0005 in molarity. The results by both methods are:

Molarity of solution with respect to Na_2CO_3	
True molarity	0.00525
As determined with HCl (average of 3 determinations)	0.00735
As determined with *p*-nitrosothymol (average of 5 readings)	0.00612

The amount of sodium carbonate in a sample of sodium bicarbonate was determined with *p*-nitrosothymol in the manner previously described. To an approximately 0.15 *M* solution of this sodium bicarbonate were added enough 0.0175 *M* sodium carbonate solution and distilled water to give a solution which was approximately 0.075 *M* with respect to sodium bicarbonate and 0.00575 *M* with respect to sodium carbonate. The sodium carbonate in aliquot portions of this solution was determined by titration with standardized hydrochloric acid, using a mixed indicator composed of thymol blue and cresol red as recommended by Simpson (*8*). Other aliquot portions were assayed for sodium carbonate, using *p*-nitrosothymol in the manner described above. Twelve color reference standards which ranged from 0.00275 to 0.00825 *M* with respect to sodium carbonate were used, and each successive tube in the series differed to the extent of 0.0005 in molarity. The results by the two methods are:

Molarity of solution with respect to Na_2CO_3	
True molarity	0.00575
As determined with HCl (average of 3 determinations)	0.00620
As determined with *p*-nitrosothymol (average of 5 readings)	0.00600

Stability of Reference Standards

The influence of aging of the reference standards on the accuracy of the method was determined.

A series of twelve reference standards which ranged from 0.00275 to 0.00825 *M* with respect to sodium carbonate, with each successive tube differing to the extent of 0.0005 in molarity, was prepared by the method described above, except that the indicator was shaken with the known carbonate solutions for 30 minutes. The reference standards were kept for 8 days, during which they were checked periodically with a carbonate solution of known molarity which was prepared just prior to the periodic comparisons by the same procedure used with the reference standards.

The results of these comparisons are:

True molarity of solution with respect to Na_2CO_3	0.00525
Molarity with respect to Na_2CO_3	
Determined with fresh standard (average of 3 determinations)	0.00537
Determined with day-old standard (average of 3 determinations)	0.00550
Determined with 3-day-old standard (average of 3 determinations)	0.00565
Determined with 5-day-old standard (average of 3 determinations)	0.00567
Determined with 8-day-old standard (average of 3 determinations)	0.00567

Discussion

While all the foregoing experiments involved the use of only sodium carbonate and sodium bicarbonate, it was found by preliminary experiments that the method could be applied to the corresponding salts of lithium and potassium.

For the *p*-nitrosothymol method to be accurate and its results reproducible, the conditions for mixing the bicarbonate solution must be kept the same as those under which the reference standards are prepared from known alkali carbonate solutions and *p*-nitrosothymol.

There was no difference in the intensity of the color produced when 1.1 or 2 times the calculated quantity of *p*-nitrosothymol was added to the alkali carbonate solutions. Addition of approximately twice the calculated quantity of *p*-nitrosothymol provides a safe margin, especially in the case of the unknowns where the carbonate content would have to be estimated.

As may be seen from the experimental data, the method is more accurate when freshly prepared reference standards are used. The reference standards change less when they are kept in a closed cupboard than when they are exposed to the light.

As was expected, boiling a solution of sodium carbonate or sodium bicarbonate with the indicator gave a darker color than when solutions of the same strength were shaken with the indicator at room temperature.

The *p*-nitrosothymol method is most accurate when only a little carbonate is present with much bicarbonate, the conditions under which the titration method is least exact.

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A Schematic Procedure for Identification of Common Commercial Plastics

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THE use of synthetic resins or plastics is daily becoming more and more important. Not only are these materials being used for original purposes, but, even more important, they are gradually replacing tremendous quantities of metals and other vital materials in war industry.

The chemist, especially in the commercial or industrial testing laboratory, has to keep up with the great strides taken by the plastics industry if he is to remain valuable in his position. Tables of the properties and characteristics of the synthetic resins are readily available (*3*) but, to the best of the author's knowledge, no method has as yet been published to aid the chemist in the identification of a particular plastic.

This article presents, in schematic form, a simple and concise procedure for the identification of the more commonly encountered synthetic resins or plastics. Not all the known plastics are considered in this procedure, but only those which are rather uncommon under ordinary circumstances have been omitted. Only those materials which one is likely to find in consumer merchandise submitted for test have been included. Naturally, with the development of new materials and the appearance of new plastics on the commercial market, additions and revisions of the method will become necessary.

A novel feature of the procedure is the rather wide application of the olfactory sense. In most cases the odors are so intense that rather cautious smelling is advised.

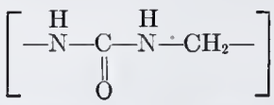
An important effect to be noted in the classification is the "green zone" around the base of the flame. This green color, when present, will be found at the area closest to the burning plastic and will be readily discernible. In all the burning tests a strip or small rod, if practicable, should be touched to the side of a small flame. If it takes fire readily, it should be removed from the flame at once; if it melts and draws away from the flame, it should be moved so that it is kept in the flame until it takes fire, but not for more than 10 seconds. When the flame of the plastic is to be examined after removal from the Bunsen flame, this should be done immediately following the removal, disregarding any burning characteristics occurring after the first 2 seconds.

To determine the odor of vapors produced, the flame should be blown out immediately upon removal from the Bunsen flame and the rising vapors should be smelled cautiously. No valid conclusions can be reached from smelling the plastic while it burns, and too much vapor will produce a deadening effect upon the olfactory organs.

In all cases it is advisable to have specimens of the various plastics in the laboratory, for comparison of the tentatively identified unknown material with a known specimen. The ideal forms in which plastics are to be examined are sheets and rods.

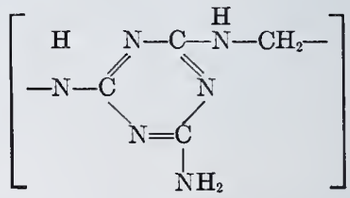
Nomenclature

Urea formaldehyde, the thermosetting polymer produced by the condensation of urea and formaldehyde, is usually glassy and infusible. Heating causes the resin to blacken and it ignites only with extreme difficulty.

Structural unit: 

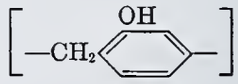
Represented by Beetleware and Plaskon

Melamine Formaldehyde, similar to urea formaldehyde in properties and appearance, is formed by the condensation of melamine and formaldehyde.

Structural unit: 

Represented by Melamac and Plaskon

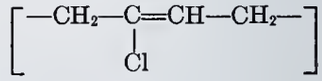
Phenol Formaldehyde, the thermosetting resin formed by the condensation of phenol and formaldehyde, is one of the most widely used of all plastics. The original work of Baekeland (*1*) describes the variation of the properties of the polymer with methods of preparation and processing.

Structural unit: 

Represented by Durite and Bakelite

Pliofilm, a thermoplastic resin produced by the addition of hydrogen chloride to the unsaturated linkage present in rubber. It is widely used in sheet form as a protective covering for various articles.

Neoprene, a rubberlike synthetic material produced by polymerization of the addition product of vinyl acetylene and hydrogen chloride. It has properties remarkably superior to those of the natural product it can replace, rubber.

Structural unit: 

Vinyl Chloride Derivatives, thermoplastic resins produced by polymerization of vinyl chloride with itself or with other compounds such as vinyl acetate. The products are elastic and may be transparent. Many of the plastic suspenders and wristwatch bands are made of this material.

Represented by Vinylite V and Koroseal

Casein. These are infusible resins produced by the processing and molding of milk protein. The products are usually opaque, but may clear if the casein is hydrolyzed first. The material is very tough.

Cellulose Acetate, a thermoplastic product produced by the reaction between acetic anhydride and cellulose, is an ester of the alcohol cellulose. Safety film is prepared from this material.

Represented by Lumarith, Tenite I, and Fibestos

Cellulose Nitrate, the resin produced by the nitration of cellulose with mixed sulfuric and nitric acids. When plasticized with camphor it is known as celluloid. It is a highly flammable material which is finding less and less use with the development of newer plastics because of this hazard.

Represented by Celluloid and Hycoloid

Methacrylate Resins, thermoplastic products formed by polymerization of one or more compounds of the type α-methyl methacrylate, methyl acrylate, ethyl methacrylate, etc. The product is readily prepared clear and colorless. The aviation industry is becoming one of the chief users of this resin. A disadvantage of the material is its comparatively low resistance to abrasive materials.

Represented by Plexiglas and Lucite

Polyvinyl Formal, Acetal, and Butyral, thermoplastic resins produced by the condensation of polyvinyl alcohol with formaldehyde, acetaldehyde, and butyraldehyde, respectively. They are not very frequently encountered in consumer goods, but

show definite promise of much wider application than they enjoy at present.

Represented by Formvar, Alvar, and Butvar

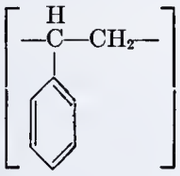
Nylon, the condensation polymer formed by the interaction of adipic acid and hexamethylenediamine. It is thermoplastic and has exceptional tensile strength. Nylon stockings, bristles, and tennis racket strings on the market illustrate the versatility of this product. Parachute manufacture consumes most of our present output.

Structural unit: $\left[-\underset{\underset{O}{\|}}{C}-(CH_2)_4-\underset{\underset{O}{\|}}{C}-(CH_2)_6-NH-\right]$

Cellulose Acetobutyrate, a thermoplastic resin produced by reacting cellulose with acetic butyric anhydride, $C_3H_7CO-O-OCCH_3$. It may be prepared in transparent sheets or in heavy opaque form. Like cellulose acetate, this product is an ester of cellulose.

Represented by Tenite II

Polystyrene, a thermoplastic material produced by polymerization of the aromatic hydrocarbon, styrene. It is therefore highly resistant to attack by inorganic acids and alkalies. It is very widely used.

Structural unit: 

Represented by Styron, Lustron, and Loalin

Ethyl Cellulose, an ether formed by the intermolecular dehydration of cellulose and ethyl alcohol. It is very similar in appearance and properties to the ester cellulose acetate.

Represented by Ethocel

Cellulose, the common transparent material widely used for wrapping packages. Strictly speaking it is not a synthetic resin, but merely reprecipitated cellulose. It is included, however, because of its close resemblance to other materials used for similar purposes.

Represented by Cellophane

Vinylidene Chloride Resins, the newly developed very resistant resins produced by the polymerization of vinylidene chloride, unsymmetrical dichloroethylene. It is produced in filament and sheet as well as tube form.

Structural unit: $[-CCl_2-CH_2-]$

Represented by Saran and Velon

Procedure

The plastic, in strip or rod form, if possible, is held at one side of a Bunsen flame until it takes fire, but not for more than 10 seconds. The following scheme is then consulted:

I. No flame is produced. The article retains its shape. The odor of formaldehyde is present in all cases.
   A. No other odor: urea formaldehyde
   B. Strong fishlike odor: melamine formaldehyde
   C. Phenolic odor: phenol formaldehyde
II. The burning plastic extinguishes itself on removal from the Bunsen flame. Specimen held just to edge of flame.
   A. A green zone is produced.
      1. Odor of burnt rubber
         a. Green area pronounced: pliofilm
         b. Green area small and overshadowed by yellow: neoprene
      2. Odor acrid but not that of burnt rubber: vinyl chloride derivative
      3. Odor sweet, heavy black ash: vinylidene chloride resins
   B. Odor of burnt milk protein: casein
   C. Sparks from flame of burning plastic and acetic odor: cellulose acetate
III. The plastic continues to burn after removal from the Bunsen flame. Flame examined for color during the first second of burning.
   A. Burning very rapid and intense white flame
      1. Odor of camphor: celluloid
      2. No camphor odor: cellulose nitrate
   B. Flame predominantly blue, may have a small white tip
      1. Very strong sweet floral fruity odor: methacrylate
      2. Odor reminiscent of burning vegetation or fresh celery. Flame is almost entirely blue. The resin is soluble in aqueous 60 per cent (by volume) hydrochloric acid (*2*): nylon
      3. Weak very slightly sweet odor: polyvinyl formal
      4. Odor of rancid butter or cheese
         a. Sparks produced from flame of burning plastic: cellulose acetobutyrate
         b. No sparks, burning smooth and steady: polyvinyl butyral
   C. Flame surrounded by bright green mantle, odor of burnt rubber: pliofilm
   D. Flame surrounded by a purple mantle, sparks and an acetic odor: polyvinyl acetal
   E. Flame yellow-white and luminous
      1. Odor of butyric acid: cellulose acetobutyrate
      2. Odor of burnt milk protein: casein
      3. Sweet floral odor (marigold), flame smoky: polystyrene
      4. Weak very slightly sweet odor: polyvinyl formal
      5. Odor of burnt paper: cellulose
      6. Odor of burnt rubber, flame weak, small green mantle overshadowed by yellow. White flashes may appear in the flame: neoprene
   F. Flame surrounded by yellow-green mantle
      1. Burns with difficulty and sparks, producing an acetic odor. The melted burning plastic, when allowed to drip into water, produces heavy black-brown foamy granules or flakes: cellulose acetate
      2. Burns readily, once started, producing a slight sweet odor. The melted burning plastic, when allowed to drip into water, produces flat disks which are light tan when the plastic is clear, or not very different in color from the original plastic when the plastic is colored: ethyl cellulose

The same resin may occupy more than one position in the scheme. This is to take into account any possible misinterpretations of the characteristics described.

Summary

A schematic procedure for identification of the commercial plastic types includes the plastics which the average chemist may encounter in his testing experiences. Seventeen families of plastics are discussed briefly, representing about one hundred plastics which are marketed under different commercial names.

Acknowledgment

The author wishes to acknowledge the valuable assistance given him by the Dow Chemical Company, the Carbide and Carbon Chemicals Corporation, and the Celluloid Corporation in sending him some of the specimens used as standards in the evolution of the method.

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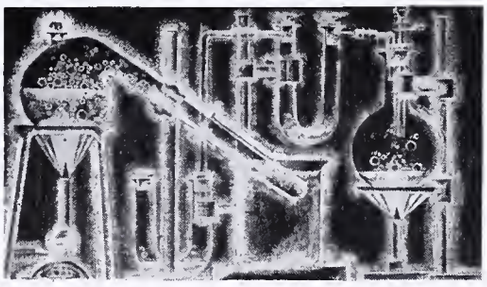

A Versatile Continuous Reading Thermionic Voltmeter

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THERMIONIC instruments for potential measurement can be roughly divided into two classes: vacuum tube voltmeters for voltage measurements in low-resistance circuits and electrometers for voltage measurements in high-resistance circuits. Few attempts have been made to combine into one instrument those characteristics necessary for high-resistance work with desirable features usually found only in vacuum tube voltmeters. The increasing use of the glass electrode and the phototube have put a premium on the development of such universal instruments.

A satisfactory laboratory voltmeter for all-purpose work should be designed with the following characteristics in mind:

Necessary	Desirable	
Low grid current	Continuous reading	Variable sensitivity
Stability	Simplicity of operation	Circuit simplicity
High sensitivity	Wide operating range	Low cost of construction
	Low drift rate	Portability

All these characteristics are not entirely compatible. The great majority of potential measuring circuits having the "necessary" characteristics have been designed around special electrometer tubes of relatively high cost or are circuits suitable only for use as null-point indicators (*4, 6, 8, 11, 13, 14, 15, 18, 22, 28, 29*). None of these instruments has enough of those characteristics listed as desirable to make it suitable for general laboratory use.

For potentiometric titration work an instrument that continuously indicates the potential is most desirable, since it allows the course of the equilibrium to be followed at all times. While several continuous-reading titrimeters have been described (*10, 12, 25, 32, 33*), few continuous-reading voltmeters suitable for high-resistance work are to be found in the literature (*16, 24, 31*). Of the circuits published, that of Penther, Rolfson, and Lykken (*24*) is the most promising for work in high-resistance systems. The principal limitation of their instrument is that it has a range of linear response of only 0.110 volt. Further voltage changes must be compensated for with a step-potentiometer. In titrations of a routine nature where speed is important, it would be more advantageous to have an instrument with a wider voltage range requiring no adjustment during the course of the titration.

The circuit described below was developed in an attempt to combine in one instrument as many of the listed features as possible.

Circuit Synthesis

Recently Garman and Droz (*10*) designed a simple vacuum tube voltmeter employing the bridge-type circuit usually found in stable electrometers. The tube used in their circuit is an ordinary pentagrid converter, type 1A7GT, which has the necessary characteristics for an electrometer tube. The 1A7GT is particularly useful, since it also has a low filament current, with resulting low heat emission and battery drain.

In the circuit of Garman and Droz the plate and anode grid of the pentagrid converter are connected as a conventional Wheatstone bridge. The voltage to be measured is impressed between the control grid and filament and results in a simultaneous increase in plate current and an equal decrease in anode grid current or vice versa. This results in doubly unbalancing the bridge. In their original circuit Garman and Droz connect a sensitive current meter across the bridge to indicate the degree of unbalance. A high-resistance meter would be desirable, to build up a maximal potential difference across the bridge, but a low-resistance meter would give a greater deflection for a given voltage difference.

Experiment showed that, in the voltmeter described, increasing the meter resistance about 90 times decreased the sensitivity only threefold, indicating that the high resistance allowed 30 times as high a voltage to be developed across the bridge as the low resistance. The ideal meter, then, should have a very high resistance as well as a high sensitivity.

The ideal meter characteristics are approached by connecting across the bridge of the Garman-Droz circuit an ordinary vacuum tube voltmeter (Figure 1). The second stage is, in effect, a volt-

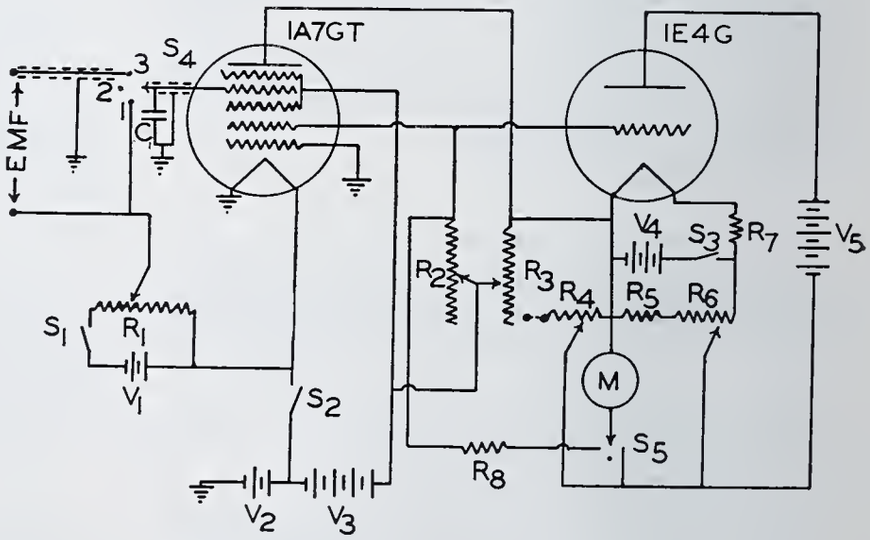

FIGURE 1. VOLTMETER CIRCUIT

C_1. Tested condenser, capacity 0.02 mfd.
M. 0–500 (120-ohm internal resistance) or 0–100 (900-ohm internal resistance) Triplett microammeter
R_1. 10,000-ohm uniform volume control
R_2. 5000-ohm uniform volume control
R_3. 10,000-ohm uniform volume control
R_4. 2000-ohm uniform volume control (for use with 0–500 microammeter) with switch cover plate for S_3
R_5. 5000-ohm 0.5-watt
R_6. 5000-ohm tapered volume control with switch cover plate for S_1 and S_2
R_7. 30-ohm 0.5-watt
R_8. 2000-ohm 0.5 watt
S_1, S_2. Double-pole single-throw switch
S_3. Single-pole single-throw switch
S_4. Special high-resistance SP3 position switch with off position in center
S_5. SP3 position switch with off position in center
V_1. 2 1.5-volt flashlight cells
V_2. 1.5-volt filament battery
V_3. 45-volt plate battery
V_4. 2 1.5-volt filament batteries
V_5. 2 45-volt plate batteries

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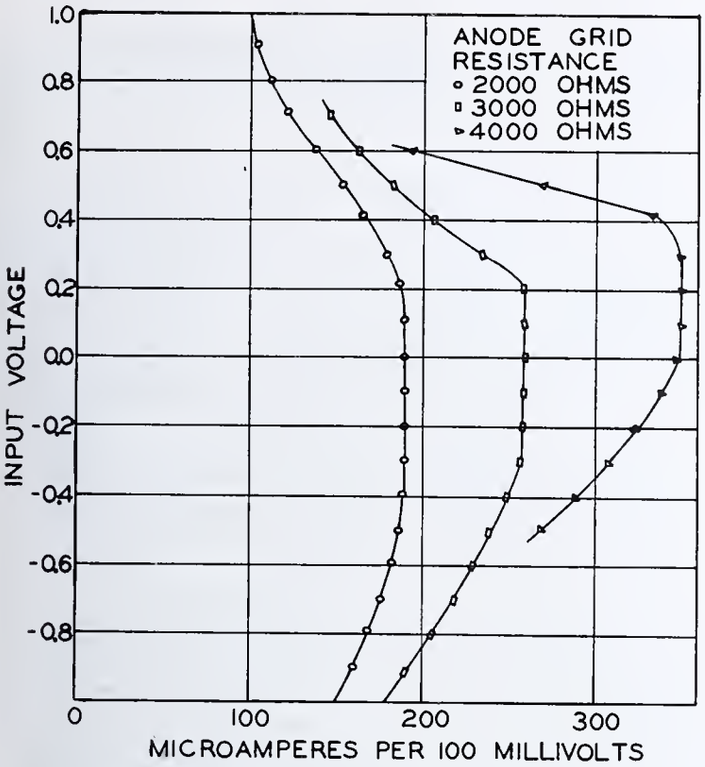

FIGURE 2. SENSITIVITY VARIATION WITH BRIDGE RESISTANCE

meter having a resistance of several megohms but with a sensitivity of about 850 microamperes per volt. The 1E4G tube was chosen for this stage because it has advantages over other voltage amplifiers in possessing a low filament current, high transconductance, zero grid bias, and the high stability inherent in triodes.

To this basic circuit were added several other features for increasing the versatility of the instrument. One of these is the potentiometer circuit, R_1-V_1, in the *EMF* input lead to the filament of the electrometer tube.

The bridge resistances, R_2 and R_3, are separate variable resistances to permit varying the instrument sensitivity as desired (see Figure 2). The top view of the instrument (Figure 3) shows the relative positions of R_2 and R_3. For operation at a given maximum sensitivity, either the plate resistance, R_3, or the anode grid resistance, R_2, may be fixed. S_5 and R_8 are included in order that the microammeter may be used for balancing the bridge.

Stadie, O'Brien, and Laug (*30*) point out the desirability of increasing the capacity of the control grid by connecting to it a suitable grounded condenser, C_1. This increased capacity serves to protect the grid potential from momentary changes due to the opening of switch S_4. In order to prevent leakage currents, this condenser must have a resistance at least as high as the internal resistance of the control grid. To reduce leakage further it is necessary to construct S_4 and the input jack in the grid lead with a high degree of insulation from ground. Many suitable switch designs have been published (*6, 13, 28, 30*). The authors have found that glass tubing makes satisfactory stand-off insulators for this purpose. These can be fastened to the chassis by means of a short bolt and de Khotinsky cement. The jack in the grid circuit was designed to take the Beckman glass electrode fittings. The electrometer tube is shielded by means of a glass cylinder fitted over the tube base and covered with a metal cap connected to ground. The shielded lead to the grid of the electrometer tube passes through this metal cap (Figure 3). The surface of the 1A7GT and the glass cylinder are covered with paraffin to prevent leakage of surface currents. The control grid lead is the only part of the circuit where extraordinary care with regard to insulation need be taken.

In the second stage there are two necessary controls. One of these, R_4, varies the over-all sensitivity by shunting the microammeter. A value of R_4 equal to about 15 times the meter resistance gives a good spread of the sensitivity control with only 6 per cent reduction in maximum sensitivity due to shunting. This reduction can also be eliminated if contact is broken at the extreme end of the shunt. Coating the end of the contact surface with an insulating material is a convenient way to do this. The other control, R_6, is in the widely used D-battery circuit (*9, 26*), which is composed of R_5 and R_6 and the 6-volt filament battery. For a given setting of R_6 a fixed amount of plate current is by-passed around the meter, thus allowing the use of a more sensitive meter. The resistance of both R_5 and R_6 should be kept high to reduce battery drain and to increase the by-passing range. Under these conditions, a small rotation of R_6 causes a large change in the amount of current by-passed. For ease of control a vernier is desirable. An ordinary planetary control with a 5 to 1 ratio has been found satisfactory. The combination of the filament and D-batteries is a commonly used method of stabilizing the circuit against changes in battery voltages (*16, 19, 25, 31*).

The entire instrument with all batteries is enclosed in a 7 × 8 × 10 inch metal cabinet. Figures 3 and 4 show the panel layout and some details of the internal arrangement.

Operation and Adjustment

The preliminary steps in the operation of the instrument are: (1) S_4 is opened (position 2, Figure 1); (2) S_1 and S_2 are closed by rotating R_6 and the rotation is continued to about the center of the range; (3) S_3 is closed by rotating R_4.

In the preliminary adjustment of the instrument it is necessary at this point to balance the bridge by varying R_2 or R_3. At least an hour should be allowed for the electrometer tube to reach equilibrium before this adjustment is made. Switch S_4 is opened and then the microammeter in the second stage is temporarily connected across the bridge by closing S_5. The plate resistance, R_3, is fixed at a convenient value and the anode grid resistance, R_2, is adjusted until the current through the meter is zero. This adjustment need only be checked occasionally after the tube has sufficiently aged. When the adjustment has been made the meter is again switched back into the second stage.

Finally, the free-grid potential is located as follows: (1) With the sensitivity considerably reduced, S_4 is connected to the internal biasing battery (position 1, Figure 1). (2) The bias is adjusted by rotating R_1 until the meter gives the same reading as with the grid floating. The sensitivity is now increased and R_1 further adjusted until no drift or deflection occurs in the meter on opening and closing S_4 to the internal bias. The needle is always kept on the scale by means of R_6.

With these preliminary adjustments completed, the instrument is ready for use. The subsequent procedure depends on the type of measurement to be made.

For potentiometric titrations with low-resistance electrodes the connection of the electrodes is immaterial. The initial electrode potential is set to the free grid with the biasing control. The needle is set either to the upper or lower end of the meter scale with the D-battery control, depending upon the direction of voltage change expected during the titration. The circuit is so connected that the microammeter needle is deflected downscale as the control grid becomes more negative.

When high-resistance electrodes such as the glass electrode are used, they are always connected to the grid lead, since one side

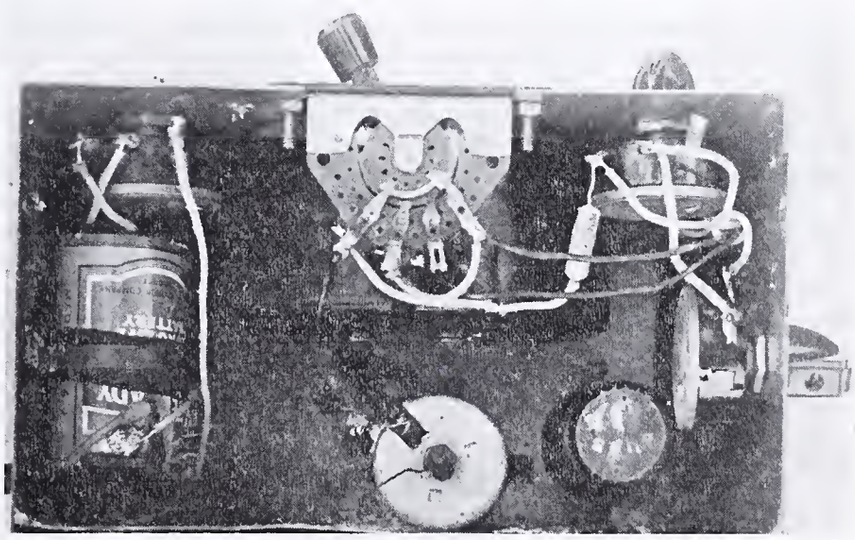

FIGURE 3. TOP VIEW OF VOLTMETER, SHOWING TUBE COMPARTMENT

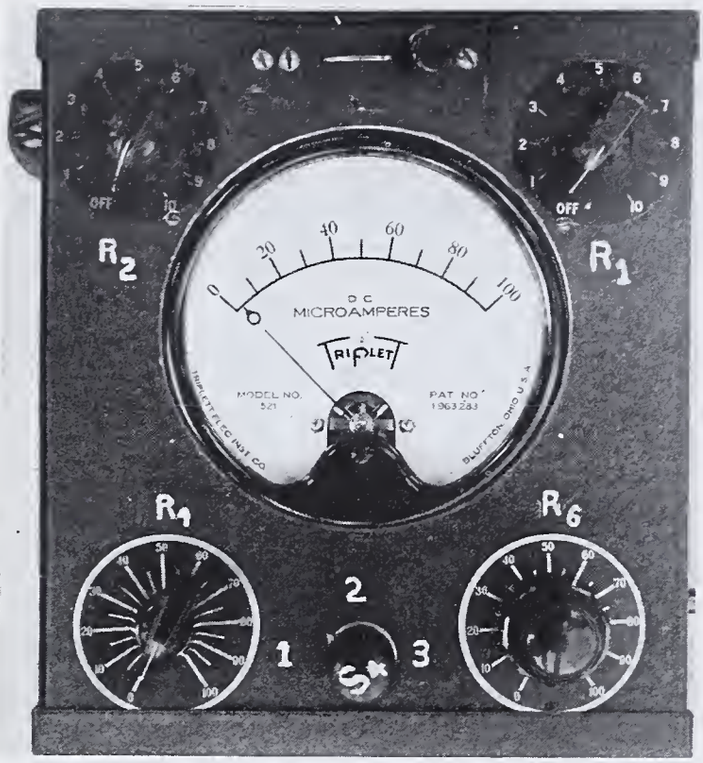

Figure 4. Front View of Voltmeter

of any high-resistance circuit must be highly shielded and common practice is to shield the glass electrode lead. The starting position of the titration will then depend on whether this electrode is to become more positive or more negative. In order to keep the grid current low it is desirable to keep the grid potential within a certain range relative to free grid, determined by the grid current characteristics of the particular tube used. To stay within this range during a titration, the needle is reset with the bias control as it approaches the end of the meter scale. This allows the use of maximum sensitivity, if desired, over a voltage range more than equivalent to 14 pH units.

If it is desired to use the instrument as a null-point indicator, it is necessary simply to adjust the grid potential with the biasing control to the free grid potential for zero input voltage and to balance out the electrode potential with a precision potentiometer inserted in the low-resistance lead.

Operating Characteristics

The grid current of the electrometer tube must be kept low for two reasons: to ensure an accurate voltage response and to prevent appreciable polarization in the electrode system (*2*). The first of these is important only in measuring circuits employing high-resistance electrodes. For a given accuracy of voltage measurment the grid current becomes the factor which limits the resistance of the electrodes that can be used.

The prevention of polarization is important for all electrode systems. According to Müller (*20*) the limiting current depends upon the type of cell and the time of current flow. In glass electrode systems, Morton (*19*) has said that currents of 10^{-10} to 10^{-9} ampere do not cause appreciable polarization. Robertson (*27*) found in working with electrodes of 2- to 3-megohm resistance that more than 6×10^{-9} ampere was required to give measurable polarization in one minute. As Figure 5 shows, grid currents of 10^{-10} ampere are never reached in the voltage range recommended for the operation of this instrument. As an additional check with the present instrument the following experiment was performed:

With a potentiometer connected in the *EMF* circuit, the potential of a glass electrode (300-megohm resistance)–calomel cell in a buffer solution was determined. The potential was then adjusted to −0.2 volt with respect to free grid. The potential of the cell as indicated by the meter reading showed no change in 30 minutes when corrected for the instrument drift. The electrodes were then disconnected for 5 minutes. When reconnected, they gave the same potential as originally measured by null-point determination. The current flowing through the cell was about 3×10^{-11} ampere. Subsequent work with the same glass electrode has shown that no measurable polarization occurs when titrations requiring several hours for completion are carried out by the recommended procedure.

The advantages of working at the free grid potential have been thoroughly discussed by Nottingham (*21*) and Rasmussen (*26*). According to these authors, the gird current at the free grid is theoretically zero. On the positive side of the free grid, the current increases sharply, owing to an electron flow to the grid. On the negative side of the free grid, the current flow is in the opposite direction. According to Metcalf and Thompson (*17*), the principal causes of this "positive" current are: (1) ionization of residual gas, (2) positive ions emitted by the filament, (3) thermionic grid emission due to heating of the grid by the filament power, (4) photoelectric grid emission due to both visible light from the filament and x-rays produced by the anode current, and (5) insulation leakages.

By the use of a high order of insulation in the grid lead (special input jack and leads, highly insulated switch S_4, tested condenser, paraffined tube surface, and desiccant) leakage currents have been minimized. The presence of one negatively charged grid and two positively charged ones between the filament and control grid will aid in screening the latter against positive ions emitted by the filament. The extremely low filament power of the 1A7G tube (0.08 watt as compared with the 0.2 to 0.5 watt given as average for electrometer tubes, *3*) should make the thermionic and photoelectric grid emissions small for this tube. Attempts to lower the plate and anode grid voltages and thus reduce gas ionization and x-ray emission resulted in drastic loss of sensitivity. Sacrifice of sensitivity was not deemed advisable, since the grid current was already low enough for most measurements.

Figure 5 shows the grid current characteristics for three 1A7GT tubes, all of which were considered satisfactory for use in the voltmeter. Out of a total of eight 1A7GT tubes tested, six Sylvania and two Ken-Rad, only three tubes were found to be suitable for use; two of these were Sylvanias and one was a Ken-Rad. The other tubes in general had a very high $\partial I_g/\partial E$, so that high currents were found very near the free grid potential. Because of this variability in tubes it is necessary to make selection of the tubes with the instrument itself. The disadvantage of having to select the tubes is one commonly encountered in tubes not specially designed for use in electrometers. Satisfactory tubes are selected by measuring the current at the free grid potential and at suitable voltage intervals on either side of the potential by observing the meter deflection with and without a 5000-megohm resistance in the grid circuit. From the voltage sensitivity and the deflection difference the current is computed by Ohm's law.

The grid current characteristics of these tubes appear to be a function of the bridge balance. Tubes which balance at approximately equal plate and anode grid resistances have high grid currents, whereas tubes having a high plate resistance (low plate current) have a much lower grid current. This current would therefore appear to be intimately related to the amount of plate current, a not unexpected result, when we consider the sources of grid currents discussed above. Unfortunately, the conditions for the highest sensitivity correspond to those where the highest grid currents are found. The free grid current for the tubes found to be suitable was less than 3×10^{-13} ampere within the limit to which the free grid potential could be located by the procedure described. With the unsatisfactory tubes it was not possible to approach the free grid potential close enough to secure as low a current. The grid currents found make the instrument unsuitable for use with circuits having a higher resistance than 30 megohms

if an accurate voltage response of 1 millivolt is desired over the operating range indicated or with a higher resistance than 300 megohms if an accuracy of 0.1 millivolt is desired for null-point measurement. This would obviate its use in mass spectrometers or phototube outfits where the resistance is 10^9 to 10^{10} ohms. However, excellent glass electrodes ranging in resistance from 2 to 30 megohms may be purchased commercially or constructed by the experimenter (*5*, *27*).

The foregoing remarks apply to the instrument as constructed. Figure 2 shows that increasing the bridge resistance causes a proportional increase in sensitivity and decrease in range of linear response. When used as a null-point indicator or a short-range voltage indicator in connection with a potentiometer this would be no disadvantage.

The choice of meter used with the instrument would depend on the use for which it is intended. For null-point detection a 0–50 microammeter or galvanometer could be used. As a titrimeter a 0–500 microammeter is satisfactory. Both a 0–100 and a 0–500 microammeter have been used in the present instrument with excellent results. Random fluctuations in the meter readings have not been observed. When dry cells are used for the filament supply there is a drift which decreases to less than 1 microampere per minute after the instrument has been turned on for about 2 hours. The authors recommend the use of storage cells for the filament supplies where practicable, as the meter drift is reduced tenfold by this practice. In all the measurements reported in this paper storage batteries were used. Many experimenters have been successful in minimizing drift by various methods of internal compensation (*1*, *3*, *7*, *23*). However, rate of drift of the present instrument remains relatively unchanged in spite of attempts of the authors to make use of such compensation methods.

An attempt also was made to construct a simple alternating current power supply to replace the batteries and thus eliminate the drift effects. When voltage regulator tubes of the glow-discharge type were used, it was found, however, that the power delivered could not be controlled within the extremely small limits necessary for complete stability. More complicated regulating circuits, such as those of Working (*34*), would probably be satisfactory if they could deliver sufficient current.

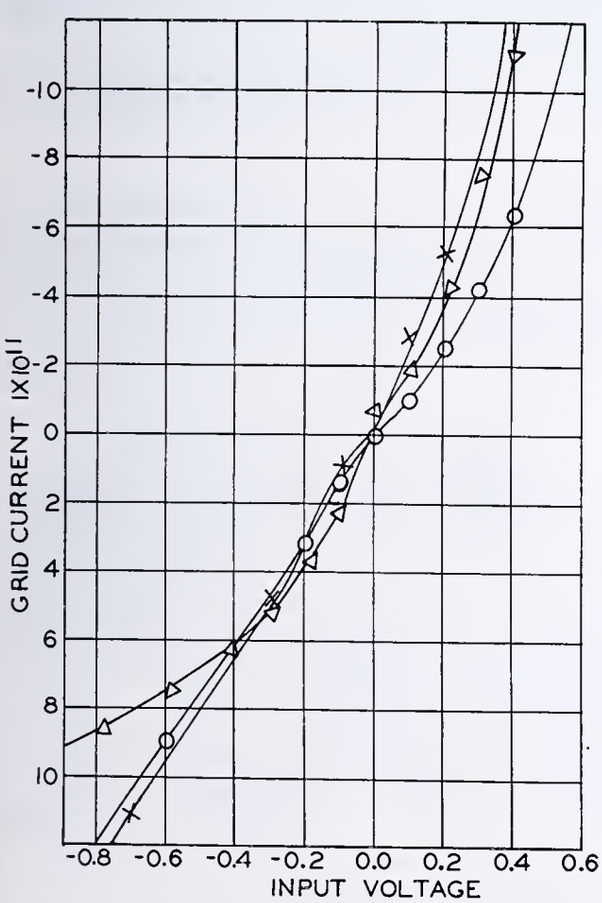

Figure 5. Variation of Grid Current with Input Voltage for Three Satisfactory Tubes

Summary

This article describes a thermionic voltmeter suitable for a wide range of laboratory measurements in both high- and low-resistance circuits.

A description of the circuit, its characteristics, and operation is given and the following uses are indicated: (1) as a direct, continuous-reading titrimeter for glass electrode systems, (2) as a direct continuous-reading titrimeter for low-resistance electrodes, and (3) as a null-point indicator in connection with a potentiometer. The instrument has proved very satisfactory in actual operation over a period of several months in each of these applications. Further applications such as the measurement of photocell currents, etc., in circuits of moderately high resistance are indicated.

The total cost of the materials used in the instrument less batteries was about $25. The particular batteries used cost $5. The low current drain ensures long life for these batteries.

Acknowledgment

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Contribution from the Scripps Institution of Oceanography, New Series, No. 189.

Simple Automatic and Recording Balance

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The paper describes the design, construction, and operating characteristics of a simple, rugged, automatic recording balance, which may be constructed in the average laboratory workshop. The instrument is suitable for most of the chemical engineering laboratory operations where a continuous record of sample weight is desired over long periods.

THE authors were faced with the problem of continuously weighing a sample of approximately 1 kg. during a drying test covering one day or more. The reduction in sample weight in most cases was about 0.5 kg. This indicated that a sensitivity of 0.1 gram would be sufficient and would lead to results accurate to 0.5 per cent with the sample weight varying as little as 20 grams.

Numerous balances are described in the literature (*1–5*). These are rather complex and expensive, but are reported to be extremely precise over a narrow range of weight. The range may be extended by the addition of counterweights but for the purpose in mind this would result in constant attendance. For the most part, the instruments described are not easily built in the average laboratory workshop by the student or mechanic available. It was therefore decided to design and construct an instrument which would be rugged, cover a wide range of weight (1 kg.), and still have a sensitivity of 0.1 gram.

In view of the desired sensitivity, compensating devices such as those employed by Müller and Garman (*3*) and Ewald (*2*) were dispensed with and the balance was greatly simplified. The instrument maintains the balance arm at equilibrium by reeling in or playing out sufficient chain to counteract changes in sample weight. The chain mechanism is actuated by an energized pointer which is located above the knife edges on the balance beam. The pointer makes contact with either of two adjustable plates set 0.5 cm. apart, which in turn are connected to relays operating the clockwise and counterclockwise circuits of a small motor. The recording mechanism is integral with the chain reel and a moving pen follows changes in weight continuously.

Balance and Recorder

The balance is mounted in the wall of the dryer and the compensating and recording mechanism is placed in such a position that the chain describes a smooth catenary curve between the end of the balance arm and the chain reel. Figure 1 illustrates the assembled apparatus. The sample is suspended from the end of the balance arm inside the dryer, the chain being attached to the other end of the arm outside the dryer. The balance arm is maintained at equilibrium by the addition to, or removal of, the chain from a reel operated by a small (1.5 kg.-cm., or 20 ounce-inch) reversible, 110-volt, alternating current motor of the shaded pole type.

The actuating circuit diagram is shown in Figure 2. The balance arm pointer is energized by connecting one pole of a 45-volt, dry cell battery to the top of one of the steel knife edges by a fine copper wire. A condenser is placed in parallel with the pointer to reduce sparking at the contacts. A slight change in weight causes the pointer to touch one of the contact plates which operates the relays energizing the clockwise or counterclockwise circuits of the motor. The contact plates are insulated from the supporting frame by thin sheets of Bakelite paper.

The motor is coupled to the shaft through a gear train which reduces the motor speed from 3600 to 1 r. p. m. at the shaft. Friction is reduced by mounting the shaft on ball bearings set in Bakelite supports. The recording drum and chain reel are mounted on the shaft by suitable setscrews and can be removed and changed with ease. This affords a wide variety of chain speeds and chart sizes.

The recording pen is drawn across the drum at a uniform rate by a silk thread which passes over a pulley attached to the hour

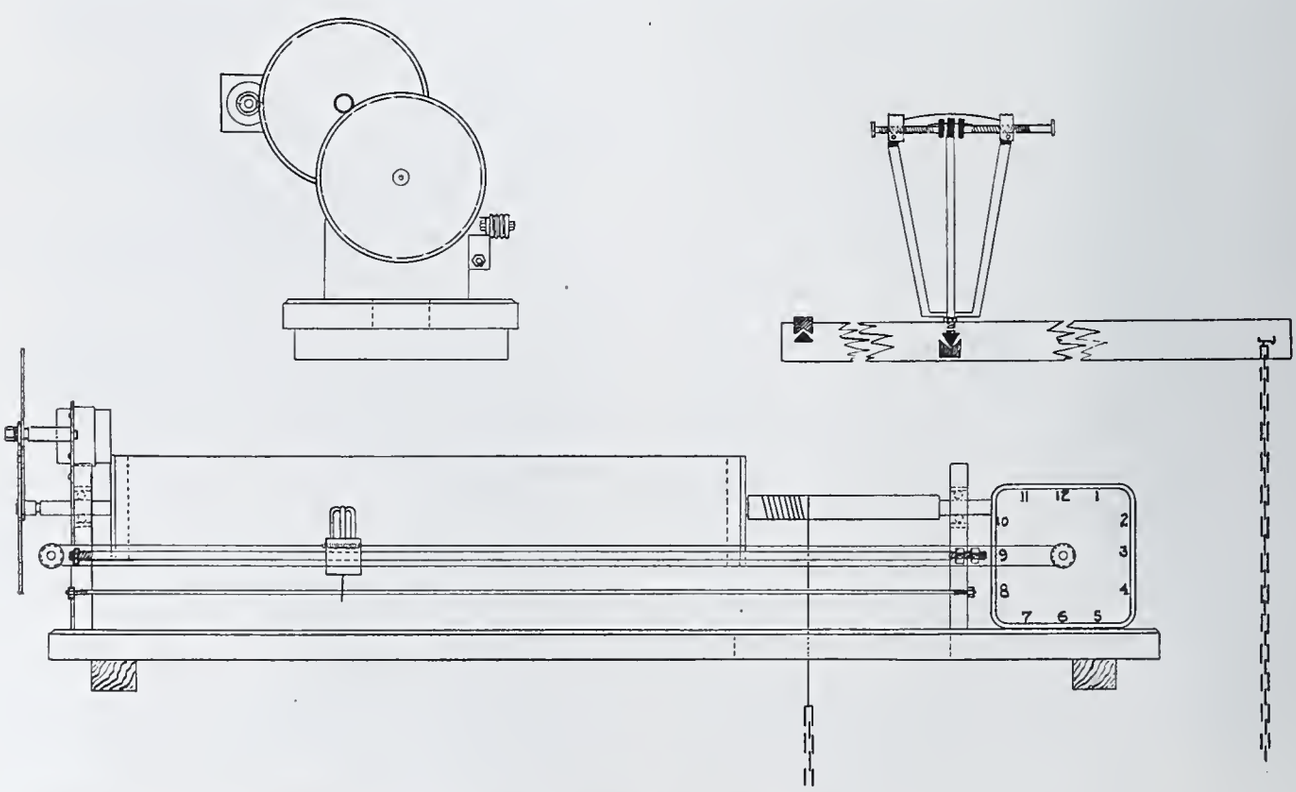

FIGURE 1. ASSEMBLED APPARATUS

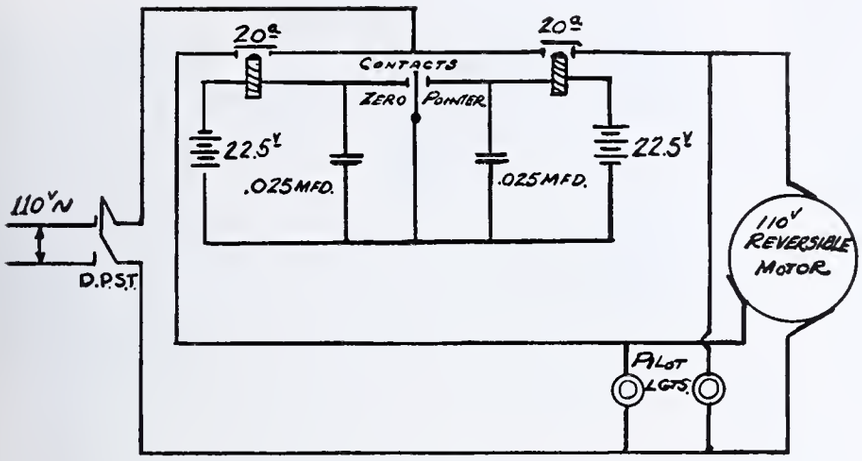

FIGURE 2. CIRCUIT DIAGRAM OF COMPENSATOR

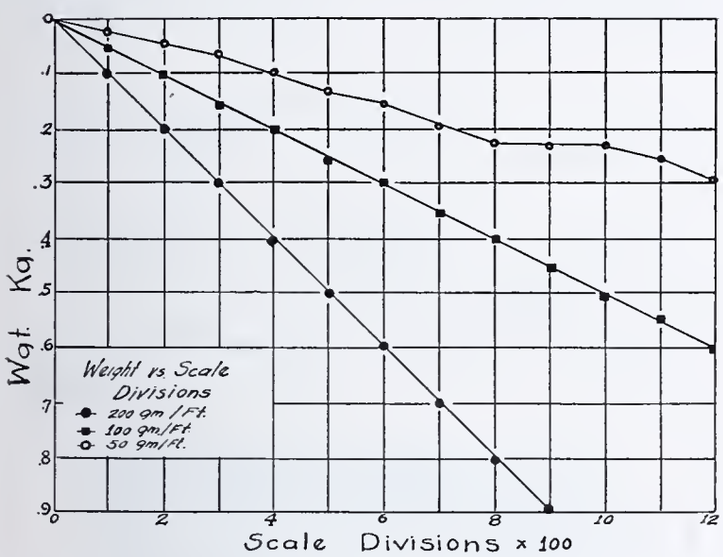

FIGURE 3. CALIBRATION CURVES OF WEIGHT AGAINST SCALE DIVISIONS

hand of an ordinary alarm clock. In order that the thread may be maintained in tension at all times, it is connected to the pen carriage by means of a small spring.

Inaccuracies in winding a chain of large size on the reel are removed by attaching a short length of silk thread to the end of chain and winding only the thread. This ensures uniform removal of the chain weight from the balance arm as the drum rotates.

Operational Characteristics

The precision attained by the instrument depends upon the choice of chain, size of recording drum, efficiency of the contact system, and sensitivity of the balance itself. A large drum and a small chain cause slight changes in weight to become appreciable in terms of the weight-time curve produced by the moving pen. The sensitivity is then dependent upon the efficiency of the activating contacts and the balance arm itself. It has been found unnecessary in this case to attain a sensitivity of better than 0.1 gram over a range of 1 kg. These limits were found to be within the over-all sensitivity range of the contact system and balance arm. The range of weight possible is limited to the available headroom for a chain of any particular size.

Hunting is reduced almost to the point of imperceptibility by proper setting of the screw adjustment of the variable contact plates. A slight amount of hunting is desirable, for it indicates that the weighing is not being made on a static beam, and the compensator is adding or removing sufficient chain to keep up with changes in sample weight.

The size of chain and drum is chosen so that each gram of sample weight corresponds in magnitude to a large scale division on the recording paper. The recording pen is moved across the paper at a rate which permits each large scale division of the paper to represent 15 minutes. This makes it easy to convert the indicated readings to terms of time and weight. By marking the position of the chain at a known weight, and setting the chain in the same position at the start of all weighings, the scale divisions of the resultant graph are made to take on absolute values.

The balance was calibrated against a standard set of analytical weights. The calibration curve of scale divisions against weight in grams is found to be a straight-line relationship over the desired range (Figure 3), provided that there are 3 or more feet of free chain hanging between the reel and balance arm. A plot of weight in grams against time indicates the desired sensitivity (Figure 4). The curves indicate that changes in weight of less than 0.1 gram are recorded by the instrument, but these were found to be unreliable, in that subsequent addition and removal of less than 0.1 gram did not result in uniform readings in all cases. With a chain

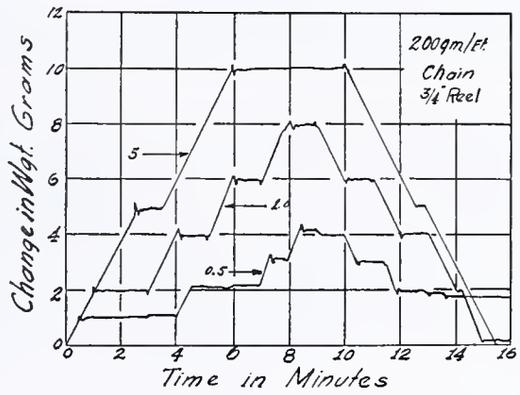

FIGURE 4. CHANGE IN WEIGHT PLOTTED AGAINST TIME REQUIRED TO REACH EQUILIBRIUM

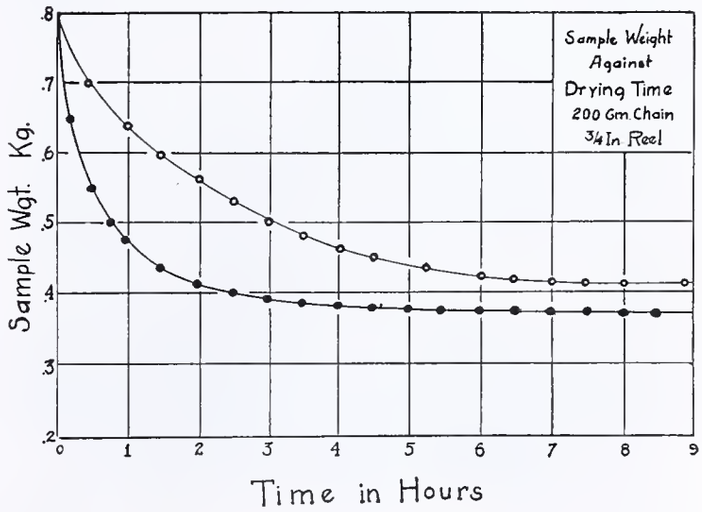

FIGURE 5. TYPICAL WEIGHT-TIME CURVES TAKEN DURING 9-HOUR DRYING TEST RUN

Table I. Cost of Construction and Material Specifications

1	A. C., 20 ounce-inch, reversible motor complete with reduction gears. Utah Radio Products Corp., Chicago, Ill., or equal	$ 3.50
2	20 ampere relays, open type. Allied Radio Corp., Chicago, Ill. Cat. No. 107, p. 150, No. 75-600 type, PRA-1 SPST, or equal	4.50
1	2 × 0.25 inch recording pen. Leeds & Northrup. Micromax recording pen or equal	2.00
2	0.5 × 0.5 × 1.5 inch ball bearings. Boston Gear Works, Boston, Mass., or equal	0.75
1	0.5 × 3 inch mild steel rod, round, hot rolled	0.35
1	3 × 24 inch brass tube, seamless. Crane Co., Chicago, Ill.	1.50
2	5-inch gear blanks, aluminum (salvage from miscellaneous shop junk)	1.60
1	1 × 6 × 12 inch Bakelite board. Allied Radio Corp., Chicago, Ill., or equal	0.75
4	Assorted chains 10 feet each in weights of approximately 50, 100, 200, and 500 grams per foot	0.81
1	Burgess 45-volt, dry cell battery with plug. Allied Radio Corp., Chicago, Ill., or equal	1.98
	Miscellaneous wire, screws, etc.	0.50
		$18.24

weighing 200 grams per foot and a chain reel 1.9 cm. (0.75 inch) in diameter, the mechanism is sensitive to 0.1 gram over a range of 1100 grams.

Large ranges of weighings will cause the drum to make more than one revolution during the experiment. This is often the case when a light-weight chain is used. The record can either be cut into sections and glued together to give one long, continuous curve, or the points of the record may be transcribed on standard 20 × 27.5 cm. (8 × 11 inch) graph paper. Figure 5 illustrates some typical curves transcribed in the second manner.

Results

The instrument has been used successfully in an investigation of the drying characteristics of various slow-drying materials. It was found to be sensitive to 0.1 gram over a range of approximately 1 kg. when using a compensating chain weighing 200 grams per 30 cm. (1 foot). The accuracy of the instrument can be improved by employing a chain of lighter weight and a larger chain reel, but this was not necessary. Nonuniformities in the lighter chains necessitated the use of a carefully made calibration curve for correcting the recorded weights. The exact starting position of the chain must be carefully set before any accurate weighings can be made with the chain weighing 50 grams per foot. This is a troublesome procedure and leads to many errors. The authors, therefore, do not recommend using a chain weighing less than 100 grams per foot. Chains of this kind always produced fairly uniform calibration curves.

Cost of Balance

The cost of the balance and accessories is small, and the largest item in the construction cost is the one of labor. The materials can be purchased from standard dealers. The entire unit cost was $70.00, of which labor is approximately 70 per cent (Table I).

Acknowledgment

The authors wish to acknowledge the valuable assistance of Max Schoenherr.

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The Dip Coater

An Instrument for Making Uniform Films by the Dip Method

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It has long been recognized that uniformity in thickness of a film of surface coating is one of the essential requisites for accurate evaluation of the film properties. The ability to reproduce uniform films of any thickness in the normal thickness range is also desirable. It has been shown (*6*) that mechanical methods are necessary for this, because uniform films cannot be reproduced consistently by hand operation of any of the regular application methods.

Arlt (*1*) describes an automatic spraying apparatus and Dunn and Baier (*3*) describe the automatic doctor blade. The spinning disk method was used by Haslam (*5*), Sanderson (*7*), and Scofield (*8*). Dipping machines have been developed by Bruins (*2*) and the New York Paint and Varnish Production Club (*6*). A general survey of the various methods is given by Gardner (*4*).

The object of the present work was to develop a simple but reliable dipping method which could be produced commercially and, therefore, made available to the surface coating industry as a standard method. Several types of apparatus were investigated to eliminate, if possible, the use of a motor.

Apparatus

The rate of fall of an object in media of different viscosities will vary with viscosity, and this may be used with suitable attachments to withdraw a panel at the different rates from a container of surface coating. Considerable difficulty was encountered in maintaining a uniform rate and reproducing results because of the relatively slow motion which was required.

An apparatus was constructed in which the container of surface coating was supported on a plunger which could be raised and lowered in a cylinder in which liquids of different viscosities were put. A definite improvement in this apparatus was made by installing a by-pass tube from the bottom to the top of the cylinder. The plunger had several holes in the disk, through which the liquid in the cylinder could flow freely when the plunger was raised, but the holes were automatically covered by a supplementary leather disk when the plunger began to lower. The rate of lowering of the plunger was then controlled by adjustment of a needle valve installed in the by-pass tube, controlling the rate of flow of the liquid from the bottom to the top of the cylinder. A low-viscosity liquid could then be used, such as kerosene, the viscosity of which is not affected seriously by normal changes in room temperature. At the low speeds required (2 to 4 inches per minute), considerable leakage developed between the leather and metal disks, so that a given speed could not be reproduced by a definite setting of the needle valve. This method would be satisfactory if metal check valves were used in the plunger disk, and the plunger and side walls of the cylinder machined with extreme accuracy.

An apparatus was then constructed similar to that shown in Figure 1, but using a clock motor. A set of Boston sprockets (Boston Gear Co.) was used to vary the speed with the standard chain drive. This type of motor has low power, but by hanging a

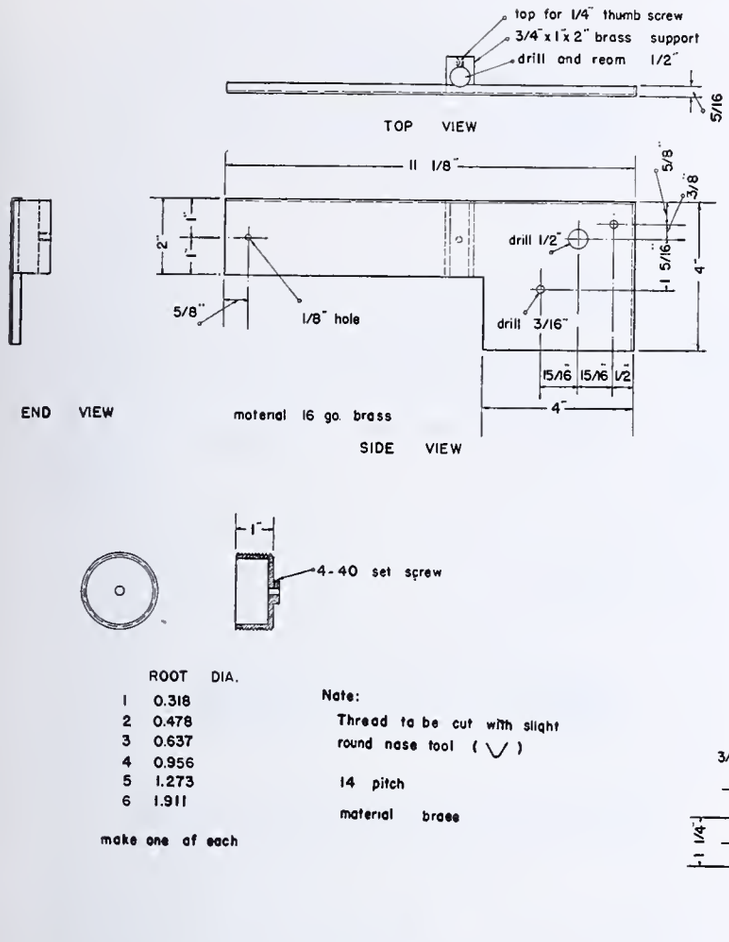
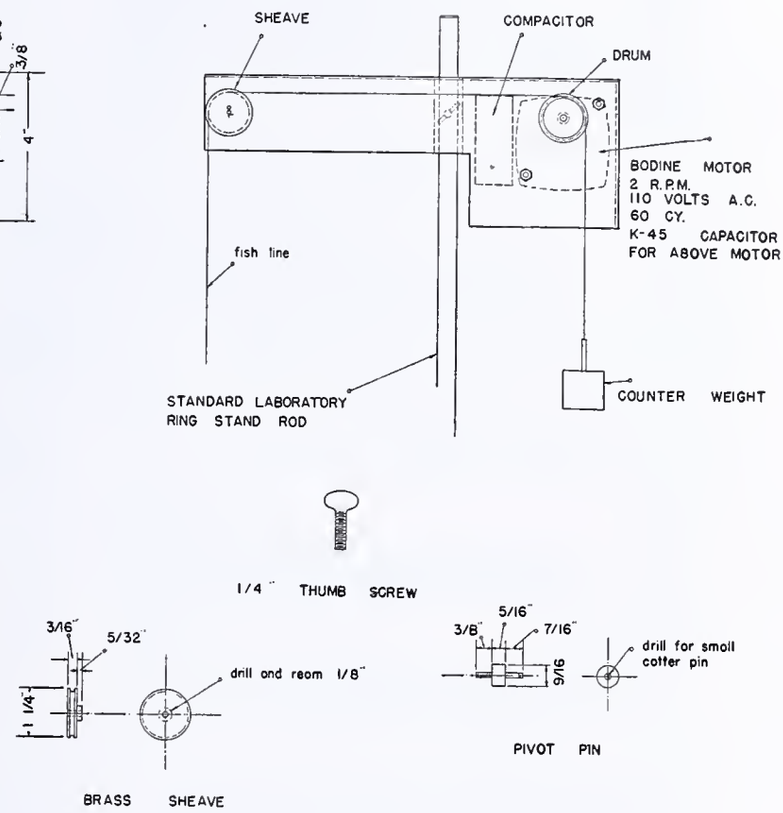


FIGURE 1. DIAGRAM OF APPARATUS FOR MAKING UNIFORM FILMS



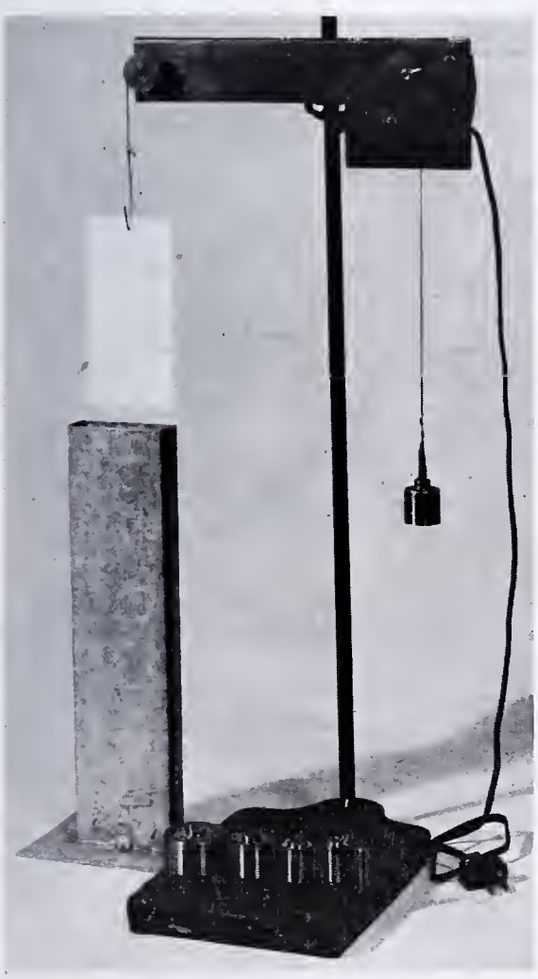


FIGURE 2

counterweight on the opposite end of the chain from the panel, practically any size of panel or number of panels could be used. This apparatus gave excellent speed control and appeared to be entirely satisfactory, but on close examination of the coated panels, a horizontal striation was found in the coating. This was caused by a slight amount of lost motion in the gear reducer of the motor. It was not due to the sprockets and chain, because when they were replaced with drum and fish line, the same striation was observed. Replacement of the clock motor with a Bodine KYC-22RC (Bodine Electric Co., Chicago, Ill.) gave results which were entirely satisfactory. A set of drums was made to give withdrawal speeds of 2, 3, 4, 6, 8, and 12 inches per

FIGURE 3

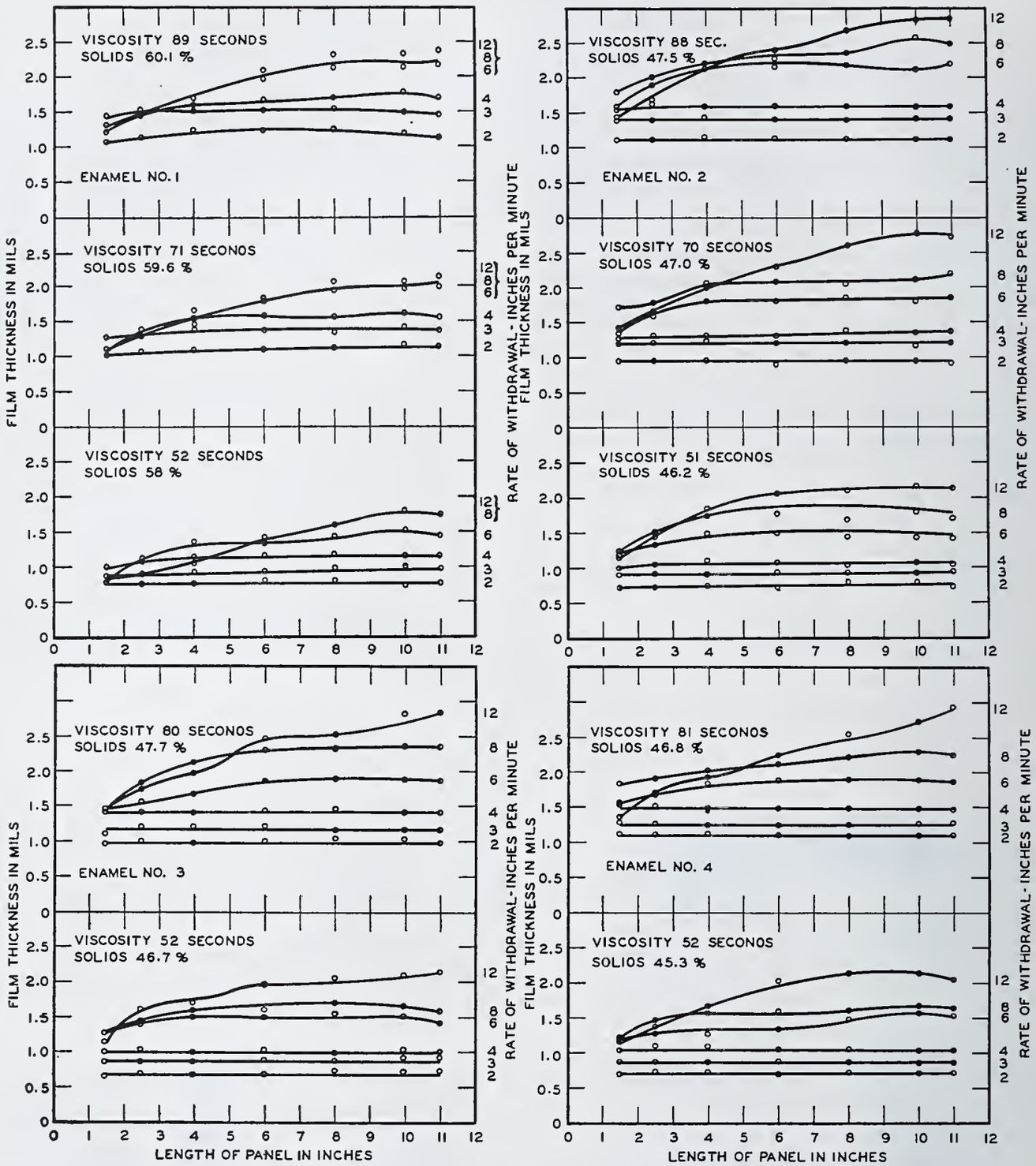


FIGURE 4. VARIATION OF FILM THICKNESS OF ENAMELS WITH VISCOSITY AND RATE OF WITHDRAWAL

minute. A fish line was given two complete winds around the drum with a counterweight hung on one end and the panel on the other end, as shown in Figures 1 and 2. The details of construction of the apparatus are shown, but arrangements have been made with the Fisher Scientific Co. for the commercial production of a similar machine, so that it will be available to those not wishing to build it.

Coating Materials

A series of four standard white baking enamels (enamels 1 to 4, inclusive) was made to determine the rate of withdrawal necessary for uniform thickness films and also the film thickness obtained with variation of viscosity of the coating material. The viscosity was measured as seconds required to flow from a No. 4 Ford cup at 78° F. in the standard manner. The materials varied considerably in ability to flow, rate of setup, and composition; but the data showed clearly that withdrawal rates of 2, 3, and 4 inches per minute gave films of uniform thickness, and the faster rates (6, 8, and 12 inches per minute) were unsatisfactory. Subsequent work was, therefore, confined to the slower rates, but in most cases a hand-dipped panel was included for comparison.

The olive drab lusterless finish and the two primers, A and B, were used to show the variation which may be expected from materials containing a higher ratio of pigment to vehicle solids than that in the enamels.

Clear coatings 1 and 2 are the same as the vehicles in enamels 1 and 2 to illustrate the effect of pigmentation. Clears 3 and 4 are typical varnishes for comparison with alkyds 1 and 2 and nitrocellulose lacquer 5. Clears 1 to 5, inclusive, are air-drying coatings, but Nos. 6 and 7 are clear-baking metal finishes, such as are used on shell cases, etc.

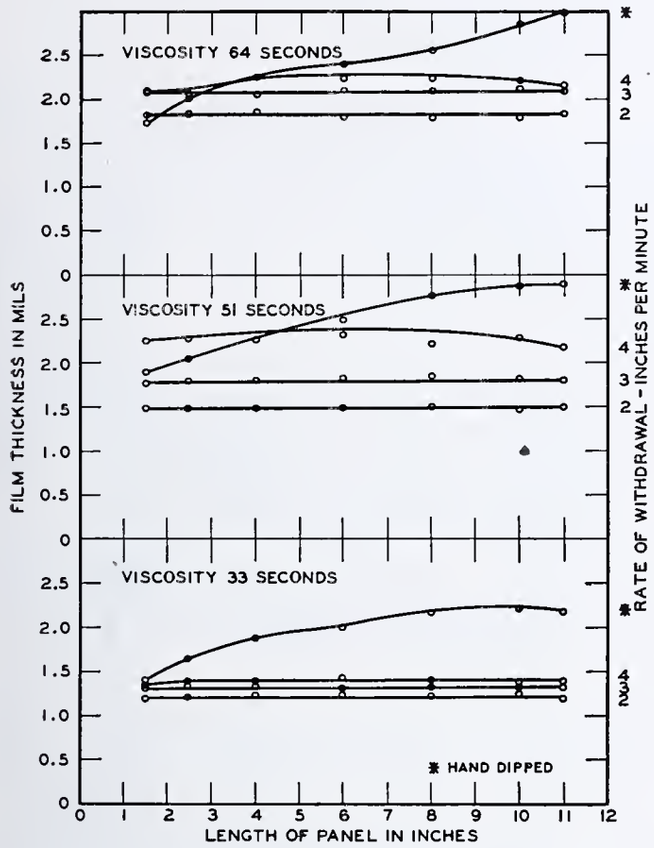


FIGURE 5. VARIATION OF FILM THICKNESS OF OLIVE DRAB LUSTERLESS WITH VISCOSITY AND RATE OF WITHDRAWAL

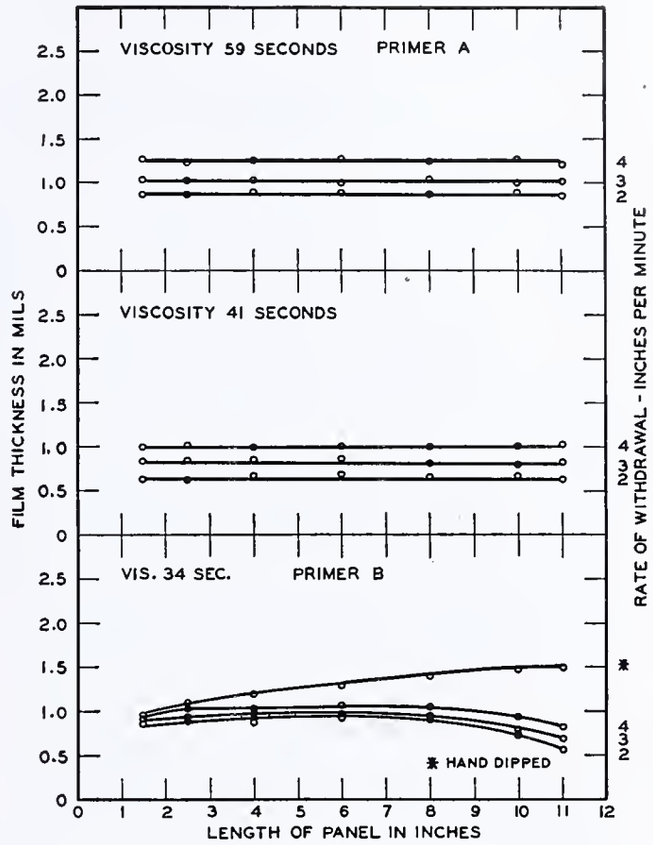


FIGURE 6. VARIATION OF FILM THICKNESS OF PRIMERS WITH VISCOSITY AND RATE OF WITHDRAWAL

TABLE I. COATING COMPOSITIONS

	White Enamels
Pigment	Titanium dioxide 100%
Vehicles	
Enamel 1	Rezyl 412–1[a]
Enamel 2	Rezyl 330–5
Enamel 3	Rezyl 330–5, 65%; Beetle, 227–8, 35%[b]
Enamel 4	Rezyl 330–5, 80%; Melmac 245–8, 20%[b]

Pigment–vehicle solid ratio, 1:1
Solvent
Enamel 1, mineral spirits
Enamels 2, 3, and 4, xylene

Olive Drab Lusterless

Pigment	% by Weight
Ferrite lemon	20
Medium chrome yellow	10
Zinc oxide	5
Raven black oxide	5
Asbestine 3X	30
Celite 349	25
Mica 270	5
Vehicle	
Rezyl 7818–1, 100%	

Pigment–vehicle solid ratio, 4:1
Solvent, mineral spirits

Primer A

Pigment	
Titanium dioxide	50
Zinc oxide	50
Vehicle	
Rezyl 412–1, 100%	

Pigment–vehicle solid ratio, 1.6:1
Solvent, mineral spirits

Primer B

Pigment	
Zinc yellow	7
Brown oxide	50
Zinc oxide	3
Celite 110	10
Asbestine 3X	20
Barytes	10
Vehicle	
Rezyl 7818–1, 100%	

Pigment–vehicle solid ratio, 2.7:1
Solvent, mineral spirits

[a] Trade mark, American Cyanamid Co.
[b] Trade mark, American Cyanamid & Chemical Corp.

DRYING SCHEDULES FOR COATINGS. The films were dried under following conditions before making film thickness measurements:

Enamels 1 and 2	Bake 60 minutes at 265° F.
Enamels 3 and 4	Bake 30 minutes at 265° F.
Olive drab lusterless	Air-dry 48 hours
Primer A	Bake 60 minutes at 325° F.
Primer B	Air-dry 48 hours
Clears 1 to 5	Air-dry 48 hours
Clears 6 and 7	Bake 15 minutes at 350° F.

TABLE II. CLEAR FINISHES

		Drier as % Metal to Total Solids	Solvent
1	Rezyl 412–1	0.04 Co	Mineral spirits
2	Rezyl 330–5	0.04 Co	Xylene
3	12.5-gallon varnish	0.04 Co, 0.04 Zn	Mineral spirits
4	35-gallon varnish	0.5 Pb, 0.1 Co	Mineral spirits
5	Nitrocellulose lacquer	.....	Solvent 10
6	Beetle 227–8, 60%; Beetle 592–8, 40%	.....	Xylene
7	Beetle 227–8, 70%; Rezyl 330–5, 30%	.....	Xylene

Composition of Clears

1 Drying oil modified alkyd, 32% phthalic anhydride
2 Drying oil modified alkyd, 41% phthalic anhydride
3 Maleic resin, drying oil varnish
100-lb. Teglac Z 152[a]
8.25-gal. oiticica oil (Cicoil)
4.25-gal. bodied linseed oil (KPO-Q)
4 Modified phenolic resin, drying oil varnish
100-lb. Phenac 604 M[b]
7-gal. treated fish oil (Celesterol)
18-gal. dehydrated castor oil (Isoline Z3)
10-gal. bodied linseed oil (KPO-A)
5 Nitrocellulose lacquer (parts by weight on solid basis)

0.5-sec. nitrocellulose	100	Dibutylphthalate	25
Rezyl 99–4	200	Blown castor oil	25

Solvent 10 (parts by volume)

Toluene	4	Butyl acetate	2
Ethyl acetate	3	Butanol	1

6 Nonoxidizing alkyd plasticized urea-formaldehyde baking finish
7 Oxidizing alkyd plasticized urea-formaldehyde baking finish

[a] Trade mark, American Cyanamid Co.
[b] Trade mark, American Cyanamid & Chemical Corp.

1, 2, 3, and 4 were heat-processed by standard procedure for varnish making.

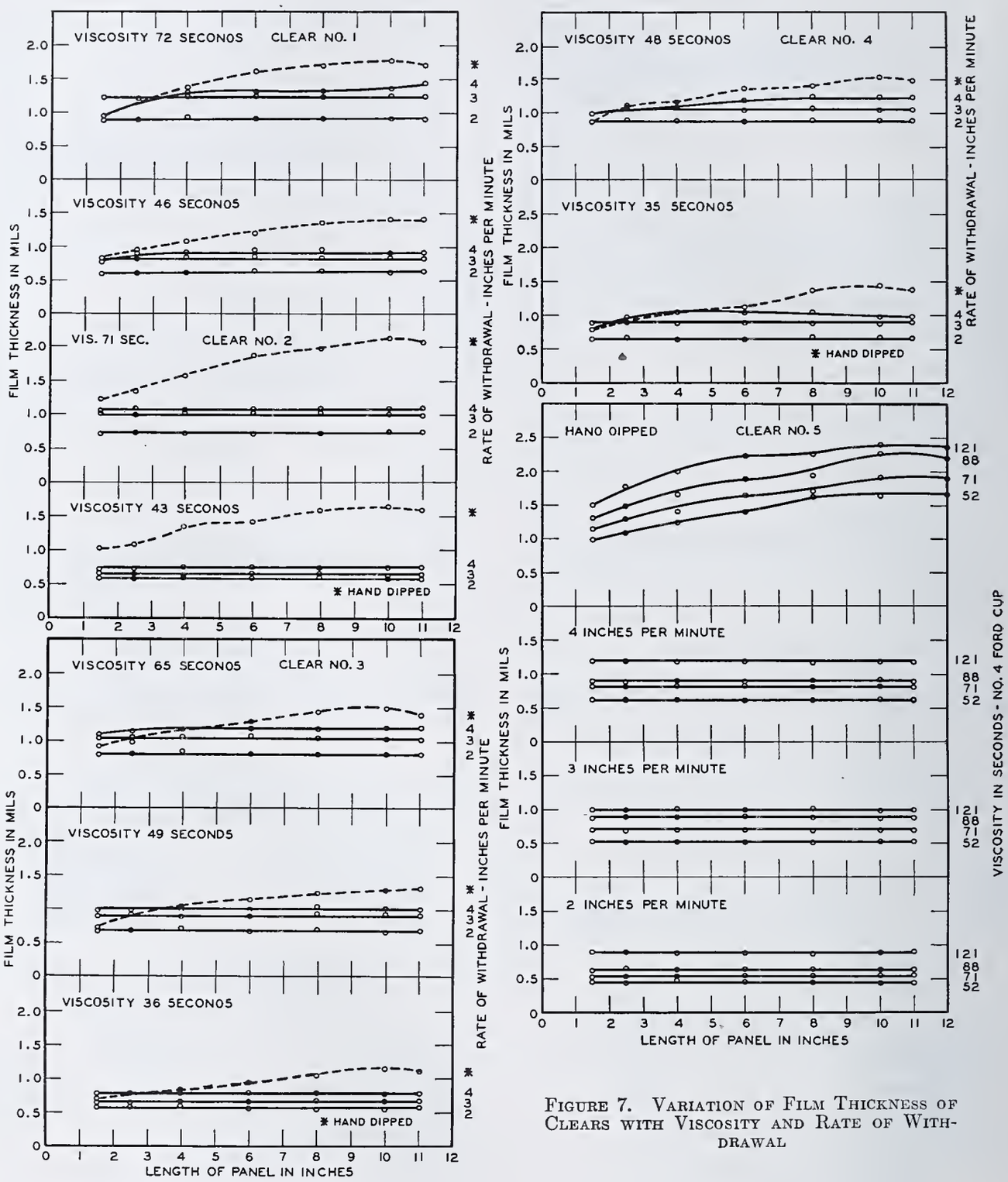


FIGURE 7. VARIATION OF FILM THICKNESS OF CLEARS WITH VISCOSITY AND RATE OF WITHDRAWAL

The air dry condition was maintained constant at 78° F., and 50 per cent relative humidity.

Procedure

All panels were dipped under conditions of constant temperature and humidity, 78° F., 50 per cent relative humidity. Cold-rolled steel panels (auto body stock) were cleaned thoroughly with toluene, and dried in a stream of compressed air free from oil or moisture. The cleaned panel was hung on a small wire hook attached to the fish line and lowered by hand into the container of surface coating to a depth of approximately 0.25 inch below the surface of the coating. The panel was then withdrawn by the dip coater at a speed dependent on the size of drum used. The drums of various size are interchangeable on the motor shaft by simple adjustment of the setscrew. The coated panels were maintained in a vertical position and dried as shown in the above schedule.

The film thickness was measured by the dial gage method (Figure 3). The coated panel was held firmly to the bed plate at four points by a special clamp shown in the figure. The total thickness (film and panel) was measured, the film removed by solvent, and the thickness of the panel determined without changing the position of the panel under the gage. The film thickness is the difference between the first and second measurements. It is extremely important that the panel be held rigidly; the clamp was designed especially for this work. It is also desirable that any slight distortion in the metal panel be eliminated. This may be done by having a threaded steel pin with a slightly rounded end protrude through the bed plate about 0.001 inch directly under the dial gage spindle. An Ames upright gage No. 13 was used in this work. The film thickness may be measured by any other method of sufficient accuracy, such as the microscope method described by Stoppel (*9*).

The film thickness was measured at seven points along the 12-inch panels with the results shown in Tables III to VII.

TABLE III. FILM THICKNESS OF ENAMELS

Inches from top	Rate of Withdrawal, Inches per Minute: 2	3	4	6	8	12
	Film thickness in mils					
	ENAMEL 1					
	Viscosity 52 seconds, No. 4 Ford cup, 58% solids					
1.5	0.78	0.86	0.96	0.81	0.86	0.88
2.5	0.75	0.90	1.10	1.10	1.01	1.20
4	0.74	0.90	1.14	1.33	1.17	1.23
6	0.77	0.93	1.12	1.33	1.38	1.41
8	0.77	0.96	1.15	1.42	1.55	1.57
10	0.74	0.96	1.12	1.50	1.76	1.73
11	0.75	0.95	1.13	1.43	1.73	1.70
	Viscosity 71 seconds, 59.6% solids					
1.5	1.01	1.26	1.07	1.05	1.10	1.16
2.5	1.05	1.32	1.37	1.32	1.30	1.38
4	1.10	1.36	1.53	1.42	1.65	1.60
6	1.08	1.35	1.57	1.78	1.80	1.77
8	1.12	1.31	1.54	2.03	1.99	1.90
10	1.13	1.37	1.61	1.94	2.06	2.02
11	1.10	1.37	1.55	2.01	2.10	2.02
	Viscosity 89 seconds, 60.1% solids					
1.5	1.05	1.41	1.24	1.21	1.29	1.27
2.5	1.11	1.54	1.50	1.47	1.43	1.44
4	1.20	1.53	1.70	1.63	1.61	1.65
6	1.20	1.53	1.65	1.79	1.84	1.98
8	1.22	1.52	1.68	1.99	1.98	2.06
10	1.17	1.49	1.76	2.29	2.10	2.14
11	1.14	1.45	1.67	2.35	2.19	2.14
	ENAMEL 2					
	Viscosity 51 seconds, 46.2% solids					
1.5	0.73	0.90	1.00	1.20	1.23	1.17
2.5	0.72	0.93	1.08	1.32	1.50	1.42
4	0.75	0.90	1.10	1.49	1.80	1.83
6	0.74	0.95	1.08	1.50	1.77	2.06
8	0.77	0.93	1.02	1.43	1.67	2.09
10	0.77	0.94	1.06	1.40	1.77	2.13
11	0.72	0.95	1.04	1.41	1.67	2.12
	Viscosity 70 seconds, 47.0% solids					
1.5	0.95	1.28	1.35	1.35	1.73	1.42
2.5	0.95	1.18	1.32	1.61	1.77	1.65
4	0.94	1.23	1.31	1.80	2.07	2.00
6	0.90	1.20	1.30	1.80	2.09	2.28
8	0.96	1.20	1.38	1.87	2.05	2.56
10	0.95	1.15	1.36	1.80	2.10	2.76
11	0.92	1.18	1.37	1.86	2.20	2.70

Inches from top	Rate of Withdrawal, Inches per Minute: 2	3	4	6	8	12
	Film thickness in mils					
	ENAMEL 2 (*Con't.*)					
	Viscosity 88 seconds, 47.5% solids					
1.5	1.08	1.40	1.54	1.60	1.82	1.48
2.5	1.08	1.38	1.63	1.92	1.85	1.70
4	1.15	1.42	1.60	2.15	2.20	2.15
6	1.13	1.39	1.61	2.16	2.30	2.38
8	1.15	1.40	1.60	2.18	2.35	2.67
10	1.12	1.42	1.59	2.10	2.57	2.82
11	1.12	1.42	1.58	2.18	2.47	2.82
	ENAMEL 3[a]					
						Hand Dipped
	Viscosity 52 seconds, 46.7% solids					
1.5	0.65	0.85	1.00	1.25	1.25	1.14	1.18
2.5	0.70	0.85	1.03	1.42	1.45	1.64	1.70
4	0.67	0.88	1.00	1.50	1.59	1.68	1.72
6	0.68	0.88	1.03	1.48	1.60	2.00	1.77
8	0.72	0.88	0.96	1.53	1.70	2.03	1.90
10	0.72	0.91	1.01	1.50	1.65	2.10	2.04
11	0.72	0.91	0.96	1.40	1.57	2.15	2.25
	Viscosity 80 seconds, 47.7% solids					
1.5	0.95	1.10	1.40	1.47	1.47	1.47	..
2.5	0.98	1.20	1.40	1.55	1.85	1.78	..
4	0.98	1.20	1.39	1.70	2.30	1.98	..
6	0.99	1.20	1.42	1.85	2.30	2.46	..
8	1.02	1.16	1.45	1.88	2.30	2.50	..
10	1.01	1.17	1.38	1.88	2.35	2.80	..
11	0.98	1.18	1.40	1.86	2.35	2.85	..
	ENAMEL 4						
							Flowed On
	Viscosity 52 seconds, 45.3% solids						
1.5	0.70	0.88	1.03	1.19	1.20	1.15	1.10	1.41
2.5	0.73	0.87	1.10	1.27	1.48	1.37	1.40	1.55
4	0.74	0.88	1.08	1.25	1.56	1.62	1.62	1.71
6	0.70	0.88	1.05	1.32	1.58	2.00	1.80	2.01
8	0.70	0.87	1.04	1.47	1.60	2.10	2.01	2.09
10	0.73	0.90	1.04	1.58	1.68	2.13	2.15	2.17
11	0.73	0.88	1.03	1.52	1.65	2.04	2.12	2.22
	Viscosity 81 seconds, 46.8% solids							
1.5	1.10	1.27	1.53	1.55	1.83	1.35	..	..
2.5	1.09	1.24	1.50	1.68	1.90	1.72	..	..
4	1.10	1.24	1.45	1.83	2.02	1.92	..	..
6	1.10	1.22	1.45	1.85	2.11	2.22	..	..
8	1.08	1.25	1.44	1.85	2.17	2.51	..	..
10	1.10	1.27	1.44	1.87	2.29	2.70	..	..
11	1.09	1.28	1.44	1.87	2.73	2.92	..	..

[a] Enamel 3. Material flowed on had practically the same thickness as hand dipped and 12 inches per minute withdrawal.

TABLE IV. FILM THICKNESS

Inches from top	Rate of Withdrawal, 2 Inches per Minute: Primer enamel Mils	Enamel Mils	Enamel bare steel[a] Mils	Diff. Mil	%	Rate of Withdrawal, 3 Inches per Minute: Primer enamel Mils	Enamel Mils	Enamel bare steel[a] Mils	Diff. Mil	%	Rate of Withdrawal, 4 Inches per Minute: Primer enamel Mils	Enamel Mils	Enamel bare steel[a] Mils	Diff. Mil	%
	ENAMEL 3 OVER PRIMER A														
	Viscosity 52 seconds, primer 1.00 mil														
1.5	2.10	1.10	0.65	0.45	69.2	2.32	1.32	0.85	0.47	55.3	2.51	1.51	1.00	0.51	51.0
2.5	2.10	1.10	0.70	0.40	57.3	2.34	1.34	0.85	0.49	57.6	2.53	1.53	1.03	0.56	54.2
4	2.08	1.08	0.67	0.41	61.2	2.35	1.35	0.88	0.47	53.4	2.58	1.58	1.00	0.58	58.0
6	2.15	1.15	0.68	0.47	69.1	2.38	1.38	0.88	0.50	56.9	2.58	1.58	1.03	0.53	51.4
8	2.02	1.02	0.72	0.30	41.7	2.37	1.37	0.88	0.49	55.6	2.54	1.54	0.96	0.58	60.4
10	2.15	1.15	0.72	0.43	59.7	2.34	1.34	0.91	0.43	47.4	2.52	1.52	1.01	0.53	52.4
11	2.07	1.07	0.72	0.45	62.5	2.37	1.37	0.91	0.46	50.8	2.46	1.46	0.96	0.50	52.0
	Viscosity 80 seconds, primer 1.00 mil														
1.5	2.45	1.45	0.95	0.50	52.6	2.74	1.74	1.10	0.64	58.0	3.06	2.06	1.40	0.66	47.0
2.5	2.52	1.52	0.98	0.54	55.0	2.76	1.76	1.20	0.54	45.0	3.12	2.12	1.40	0.72	51.4
4	2.46	1.46	0.98	0.48	48.0	2.79	1.79	1.20	0.59	49.1	3.05	2.05	1.39	0.66	47.5
6	2.47	1.47	0.99	0.48	48.5	2.75	1.75	1.20	0.55	45.7	3.08	2.08	1.42	0.66	46.5
8	2.43	1.43	1.02	0.41	40.1	2.76	1.76	1.16	0.60	51.6	3.10	2.10	1.45	0.65	44.9
10	2.43	1.43	1.01	0.42	41.5	2.77	1.77	1.17	0.60	51.2	3.07	2.07	1.38	0.69	50.0
11	2.45	1.45	0.98	0.47	48.0	2.80	1.80	1.18	0.62	52.5	3.11	2.11	1.40	0.71	50.7
	ENAMEL 4 OVER PRIMER A														
	Viscosity 52 seconds, primer 1.00 mil														
1.5	1.84	0.84	0.70[b]	0.14	20.0	2.02	1.02	0.88[b]	0.14	15.9	2.32	1.32	1.03[b]	0.29	28.0
2.5	1.84	0.84	0.73	0.11	15.1	2.06	1.06	0.87	0.19	21.9	2.38	1.38	1.10	0.28	25.5
4	1.85	0.85	0.74	0.11	14.9	2.00	1.00	0.88	0.12	13.6	2.35	1.35	1.08	0.27	25.0
6	1.83	0.83	0.70	0.13	18.6	2.02	1.02	0.88	0.14	15.9	2.36	1.36	1.05	0.31	29.4
8	1.87	0.87	0.70	0.17	24.3	2.03	1.03	0.87	0.16	18.4	2.32	1.32	1.04	0.28	26.9
10	1.86	0.86	0.73	0.13	17.8	2.02	1.02	0.90	0.12	13.4	2.36	1.36	1.04	0.32	30.6
11	1.85	0.85	0.73	0.12	16.5	2.05	1.05	0.88	0.17	19.3	2.32	1.32	1.03	0.29	28.2
	Viscosity 81 seconds, primer 1.00 mil														
1.5	2.18	1.18	1.10	0.08	7.3	2.73	1.73	1.27	0.46	36.1	2.96	1.96	1.53	0.43	28.1
2.5	2.18	1.18	1.09	0.09	8.2	2.78	1.78	1.24	0.56	45.0	2.96	1.96	1.50	0.46	30.6
4	2.22	1.22	1.10	0.12	10.9	2.76	1.76	1.24	0.52	42.0	2.97	1.97	1.45	0.52	35.8
6	2.20	1.20	1.10	0.10	10.0	2.79	1.79	1.22	0.57	46.7	2.98	1.98	1.45	0.53	36.4
8	2.20	1.20	1.08	0.12	11.1	2.75	1.75	1.25	0.50	40.0	3.02	2.02	1.44	0.57	39.5
10	2.20	1.20	1.10	0.10	10.0	2.76	1.76	1.27	0.49	38.6	2.98	1.98	1.44	0.54	37.5
11	2.20	1.20	1.09	0.11	10.1	2.74	1.74	1.28	0.46	36.0	2.96	1.96	1.44	0.52	36.2

[a] Values taken from enamel 3, Table III. [b] Values taken from enamel 4, Table III.

Table V. Film Thickness of Olive Drab Lusterless

Inches from top	Film Thickness: Rate of withdrawal, inches per minute 2	3	4	Hand dipped
	Mils	*Mils*	*Mils*	*Mils*
		Viscosity 33 seconds		
1.5	1.20	1.31	1.34	1.42
2.5	1.21	1.35	1.36	1.64
4	1.24	1.33	1.38	1.88
6	1.25	1.34	1.44	2.00
8	1.24	1.33	1.41	2.17
10	1.25	1.34	1.38	2.21
11	1.20	1.34	1.38	2.17
		Viscosity 51 seconds		
1.5	1.48	1.79	2.25	1.89
2.5	1.50	1.82	2.28	2.05
4	1.50	1.80	2.27	2.21
6	1.52	1.84	2.33	2.50
8	1.52	1.86	2.22	2.77
10	1.48	1.82	2.28	2.89
11	1.49	1.80	2.16	2.90
		Viscosity 64 seconds		
1.5	1.83	2.08	2.07	1.72
2.5	1.85	2.08	2.08	2.04
4	1.87	2.04	2.24	2.28
6	1.82	2.10	2.25	2.42
8	1.81	2.10	2.26	2.82
10	1.80	2.13	2.20	2.86
11	1.82	2.10	2.12	3.00

Table VI. Film Thickness of Primers

Inches from top	Rate of withdrawal, inches per minute 2	3	4	Hand dipped
	Mil	*Mils*	*Mils*	*Mils*
		Primer A		
		Viscosity 41 seconds, 58.3% solids		
1.5	0.63	0.83	0.98	
2.5	0.63	0.85	1.02	
4	0.67	0.86	1.00	
6	0.68	0.86	0.99	
8	0.66	0.80	1.00	
10	0.67	0.79	1.01	
11	0.63	0.83	1.03	
		Viscosity 59 seconds		
1.5	0.85	1.02	1.26	
2.5	0.88	1.02	1.23	
4	0.90	1.02	1.25	
6	0.88	1.00	1.26	
8	0.86	1.05	1.25	
10	0.89	1.00	1.27	
11	0.86	1.02	1.22	
		Primer B		
		Viscosity 34 seconds		
1.5	0.84	0.89	0.95	0.87
2.5	0.90	0.95	1.05	1.08
4	0.86	0.98	1.03	1.18
6	0.92	0.98	1.07	1.29
8	0.90	0.93	1.05	1.38
10	0.72	0.78	0.94	1.47
11	0.58	0.69	0.84	1.50

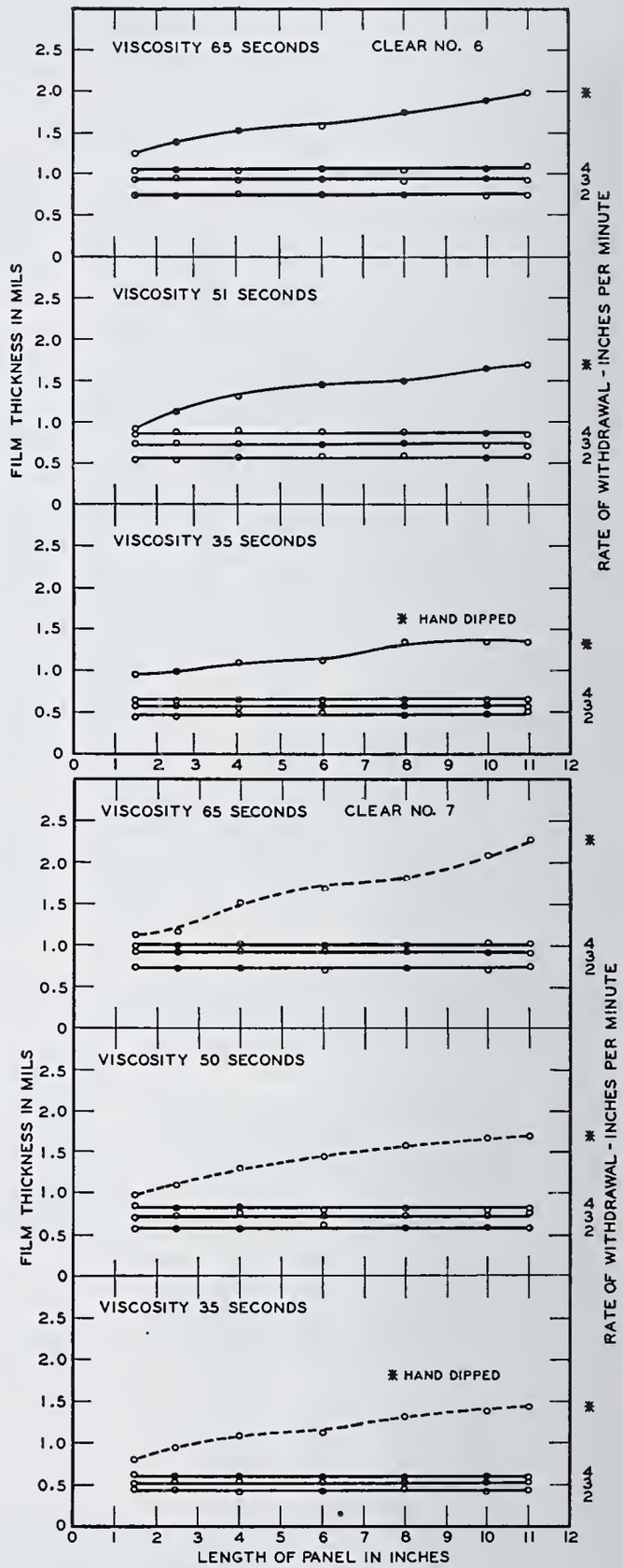


Figure 8. Variation of Film Thickness of Clears with Viscosity and Rate of Withdrawal

Discussion of Data

The results with enamels 1 to 4 show clearly that withdrawal speeds greater than 4 inches per minute do not produce uniform films. Enamel 1, with mineral spirits solvent and slower setting than the others, is not entirely uniform at the 4-minute rate nor at the high viscosity. Viscosity is much more important than the per cent of solids in determining the film thickness.

Comparison of enamel 1 (71 seconds) and clear 1 (72 seconds), having the same vehicle, shows that approximately equal film thickness is produced under the same conditions of viscosity and rate of withdrawal. The greater irregularity of the clear films at 4-inch withdrawal rate is evidence of the better flowing quality of the clear unpigmented material. Primer A, having the same vehicle but a greater pigment ratio, has less flowing quality than either enamel 1 or clear 1; therefore uniform films are obtained at the 4-inch rate. Highly pigmented coatings tend to become plastic or thixotropic with very little viscous flow. It then becomes increasingly difficult to produce uniform films by the dip method. The decreased flow may be overcome partially by lowering the consistency, but this has definite limitations. The olive drab lusterless, with considerably greater pigment ratio and different type of pigment, requires lower viscoisty for uniform films of normal thickness. The pigment ratio in primer B is less than the olive drab, but this pigment has a definite tendency to flocculate, resulting in thixotropic consistency and

TABLE VII. FILM THICKNESS OF CLEARS

Inches from top	Rate of Withdrawal Inches per Minute 2	3	4	Hand dipped
	Mil	*Mils*	*Mils*	*Mils*
Clear 1				
Viscosity 46 seconds				
1.5	0.60	0.82	0.79	0.79
2.5	0.62	0.82	0.90	0.94
4	0.64	0.85	0.92	1.04
6	0.65	0.86	0.95	1.21
8	0.64	0.83	0.96	1.36
10	0.61	0.82	0.90	1.40
11	0.63	0.83	0.91	1.40
Viscosity 72 seconds				
1.5	0.89	1.23	0.92	0.99
2.5	0.89	1.20	1.15	1.12
4	0.94	1.22	1.30	1.30
6	0.90	1.25	1.27	1.60
8	0.86	1.23	1.32	1.68
10	0.89	1.25	1.34	1.78
11	0.88	1.23	1.42	1.70
Clear 2				
Viscosity 43 seconds				
1.5	0.60	0.67	0.73	1.02
2.5	0.59	0.66	0.72	1.07
4	0.63	0.64	0.78	1.35
6	0.60	0.65	0.76	1.43
8	0.61	0.66	0.75	1.61
10	0.60	0.61	0.74	1.64
11	0.60	0.62	0.75	1.60
Viscosity 71 seconds				
1.5	0.74	1.05	1.09	1.25
2.5	0.75	1.02	1.10	1.36
4	0.74	1.04	1.09	1.61
6	0.73	1.04	1.10	1.90
8	0.74	1.05	1.09	1.99
10	0.76	1.02	1.08	2.15
11	0.76	1.00	1.10	2.08

Inches from top	Rate of Withdrawal, Inches per Minute 2	3	4	Hand dipped
	Mil	*Mils*	*Mils*	*Mils*
Clear 3				
Viscosity 36 seconds				
1.5	0.59	0.65	0.75	0.72
2.5	0.60	0.65	0.78	0.80
4	0.60	0.67	0.80	0.85
6	0.57	0.68	0.80	0.96
8	0.55	0.70	0.79	1.06
10	0.55	0.67	0.76	1.15
11	0.57	0.66	0.77	1.10
Viscosity 49 seconds				
1.5	0.67	0.91	0.99	0.73
2.5	0.69	0.91	0.99	0.89
4	0.72	0.90	1.00	1.04
6	0.68	0.90	1.02	1.15
8	0.70	0.95	1.05	1.24
10	0.68	0.95	1.03	1.30
11	0.70	0.93	1.02	1.34
Viscosity 65 seconds				
1.5	0.81	1.03	1.10	0.93
2.5	0.83	1.07	1.15	0.98
4	0.85	1.07	1.18	1.10
6	0.82	1.07	1.20	1.30
8	0.81	1.06	1.20	1.45
10	0.82	1.05	1.23	1.49
11	0.80	1.03	1.20	1.40
Clear 4				
Viscosity 35 seconds				
1.5	0.64	0.90	0.80	0.80
2.5	0.67	0.90	0.97	0.92
4	0.66	0.89	1.06	1.08
6	0.68	0.90	1.04	1.15
8	0.70	0.88	1.03	1.38
10	0.70	0.87	0.98	1.44
11	0.69	0.90	0.99	1.40
Viscosity 48 seconds				
1.5	0.86	0.99	0.98	0.87
2.5	0.90	1.09	1.05	1.11
4	0.89	1.09	1.10	1.18
6	0.86	1.05	1.20	1.37
8	0.90	1.07	1.25	1.41
10	0.89	1.06	1.25	1.54
11	0.88	1.06	1.25	1.48

Inches from top	Rate of Withdrawal Inches per Minute 2	3	4	Hand dipped
	Mil	*Mils*	*Mils*	*Mils*
Clear 5				
Viscosity 52 seconds				
1.5	0.46	0.52	0.63	0.99
2.5	0.45	0.53	0.62	1.10
4	0.48	0.52	0.63	1.24
6	0.46	0.53	0.61	1.41
8	0.44	0.52	0.61	1.63
10	0.44	0.53	0.64	1.64
11	0.44	0.54	0.61	1.65
Viscosity 71 seconds				
1.5	0.55	0.70	0.83	1.15
2.5	0.55	0.70	0.83	1.29
4	0.54	0.70	0.83	1.41
6	0.56	0.69	0.84	1.65
8	0.56	0.68	0.83	1.70
10	0.55	0.70	0.82	1.90
11	0.55	0.70	0.82	1.90
Viscosity 88 seconds				
1.5	0.64	0.87	0.90	1.33
2.5	0.65	0.90	0.90	1.50
4	0.63	0.89	0.91	1.66
6	0.65	0.90	0.90	1.87
8	0.65	0.87	0.91	1.93
10	0.65	0.86	0.92	2.27
11	0.65	0.88	0.90	2.20
Viscosity 121 seconds				
1.5	0.90	1.00	1.22	1.50
2.5	0.90	1.00	1.20	1.77
4	0.89	1.02	1.20	2.00
6	0.88	1.00	1.19	2.23
8	0.87	1.01	1.18	2.50
10	0.89	0.98	1.19	2.65
11	0.90	0.98	1.17	2.60

Inches from top	Rate of Withdrawal, Inches per Minute 2	3	4	Hand dipped
	Mil	*Mil*	*Mils*	*Mils*
Clear 6				
Viscosity 35 seconds				
1.5	0.55	0.59	0.66	0.95
2.5	0.46	0.58	0.64	0.98
4	0.50	0.57	0.66	1.10
6	0.50	0.59	0.64	1.12
8	0.50	0.58	0.66	1.35
10	0.50	0.60	0.65	1.35
11	0.50	0.57	0.65	1.34
Viscosity 51 Seconds				
1.5	0.55	0.74	0.84	0.93
2.5	0.55	0.75	0.89	1.12
4	0.58	0.75	0.90	1.31
6	0.62	0.73	0.88	1.46
8	0.60	0.75	0.88	1.49
10	0.56	0.72	0.86	1.66
11	0.60	0.70	0.85	1.68
Viscosity 65 seconds				
1.5	0.74	0.95	1.05	1.25
2.5	0.75	0.95	1.06	1.40
4	0.78	0.92	1.04	1.53
6	0.76	0.93	1.07	1.57
8	0.76	0.92	1.05	1.75
10	0.74	0.95	1.07	1.88
11	0.75	0.92	1.10	1.98
Clear 7				
Viscosity 35 seconds				
1.5	0.46	0.53	0.63	0.81
2.5	0.45	0.55	0.59	0.96
4	0.42	0.56	0.60	1.10
6	0.42	0.54	0.59	1.13
8	0.48	0.55	0.58	1.33
10	0.41	0.53	0.59	1.37
11	0.43	0.53	0.59	1.44
Viscosity 50 seconds				
1.5	0.60	0.73	0.86	1.00
2.5	0.59	0.75	0.82	1.11
4	0.59	0.77	0.84	1.31
6	0.63	0.74	0.81	1.47
8	0.60	0.75	0.79	1.59
10	0.60	0.75	0.82	1.67
11	0.60	0.78	0.82	1.70
Viscosity 65 seconds				
1.5	0.73	0.93	1.00	1.12
2.5	0.72	0.92	1.00	1.17
4	0.73	0.92	1.02	1.52
6	0.71	0.92	1.01	1.69
8	0.72	0.92	0.98	1.80
10	0.71	0.92	1.02	2.08
11	0.75	0.91	1.03	2.28

irregularity in film thickness. This material is not entirely satisfactory for dip coating and shows that the type of pigment and degree of dispersion must be considered to obtain satisfactory results.

The extreme variation in film uniformity on the panels which were hand-dipped makes this method very unsatisfactory for making test panels. The effect of slow setting produced by mineral spirits may be seen again in a comparison of clears 1 and 2. It is particularly evident in the hand-dipped panels. Clears 3 and 4 are both thinned with mineral spirits, but No. 4 is slower setting than No. 3 because of a greater ratio of oil to resin. The greater irregularity of No. 4 at comparable viscosities and the 4-inch rate of withdrawal is at once apparent. The extreme opposite to this is the fast-setting clear No. 5, with which very uniform films were obtained at the 4-inch rate even at high viscosity. However, uniform films were not obtained with this material by the hand-dipped method.

Specification ASX-736 for shell case finishes requires a film thickness of 0.6 ± 0.1 mil. Clears 6 and 7 were tested for this specification, and an examination of Figures 4 to 8 will show at once the viscosity and rate of withdrawal necessary for the desired film thickness. A series of films could be prepared by the dip coater covering a range of film thickness for a study of variation of film properties with thickness. In this way the optimum film thickness could be determined.

This work was done under conditions of constant temperature and humidity (78° F., 50 per cent relative humidity). It is well known that viscosity changes appreciably with temperature, and the temperature coefficient is not the same for a variety of coating materials. Where constant temperature is not available, it would be desirable to determine the viscosity of the coating under the same conditions as application and immediately before using. The rate of evaporation of the solvent from the film and consequently the setting time of the film is affected by the humidity of the atmosphere, but this effect is not so great as the temperature-viscosity factor and may be neglected over the normal humidity changes.

Summary

Surface coating films of uniform thickness may be made by the dip coater on steel panels for test purposes.

Film thickness may be varied over the normal useful range by changing the viscosity of the coating, the rate of withdrawal, or both. The range of rate of withdrawal for a wide variety of surface coatings is from 2 to 4 inches per minute.

Viscosity is more important than per cent solids in determining film thickness at a given rate of withdrawal.

There is an optimum flowing quality for the production of uniform films over the normal thickness range. Coating materials with very poor flowing quality are not satisfactory for application by the dip method.

The data obtained indicate the range of viscosity and rate of withdrawal necessary for surface coatings suitable for dip application to give a desired film thickness. The actual conditions of application should be determined for any specific coating and film thickness.

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Effect of Ethanol Concentration on Purity of Potassium Chloroplatinate

In Determination of Potash in Fertilizers

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FOR some time the question of the proper concentration of ethanol to be used for the determination of potash in fertilizers has been under consideration.

Ford and Hughes (*2*) reported that higher potash values were obtained with 95 per cent ethanol than when 80 per cent ethanol was employed as directed by the A. O. A. C. method for potash in fertilizers (*1*). Since this work was published, it has been suggested that perhaps the higher values were due to differences in the composition of the precipitate of potassium chloroplatinate rather than to decreased solubility of potassium chloroplatinate in the higher percentage of the ethanol. It was the purpose of this work to investigate the possibility of changes in the composition of the potassium chloroplatinate precipitate when various concentrations of ethanol are used.

Table I. Effect of Ethanol Concentration on Potash Value of a Potassium Chloride Solution

Ethanol Concentration	K_2PtCl_6[a]
%	*Gram*
80	0.2017
85	0.2034
90	0.2047
95	0.2052

[a] Averages of 8 determinations.

Procedure

The work of Ford and Hughes was extended to include potash determinations using 85 and 90 per cent as well as 80 and 95 per cent ethanol and acid-ethanol. These determinations were made on a solution of pure potassium chloride without evaporation and ignition. The temperature (20° C.) and the volume of ethanol (160 ml.) used were kept constant in all cases. The results obtained are presented in Table I.

Sufficient quantities of potassium chloroplatinate were prepared as above, using the four concentrations of ethanol, so that approximately 1 gram of each could be dissolved in water to make 500 ml. of solution, and 25-ml. portions were placed in weighed platinum dishes. Platinum reduction was accomplished by a modification of the method proposed by Swisher and Hummel (*3*) by adding 1 ml. of formic acid to each dish, heating to boiling, and transferring to a steam bath for 10 minutes. The solutions were filtered through weighed Gooch crucibles without attempting to remove all the platinum black from the dishes. The crucibles had previously been padded with a small disk of Whatman No. 5 filter paper and then with asbestos. After drying at 110° C., both the dishes and the crucibles were reweighed.

The filtrates from the platinum determination were acidified with nitric acid and chloride was determined gravimetrically by precipitating with silver nitrate. The platinum and chloride values are presented in Table II.

Table II. Effect of Ethanol Concentration on Composition of Potassium Chloroplatinate Precipitate

Ethanol Concentration	Platinum			Chloride		
	Found	Theoretical	Difference	Found	Theoretical	Difference
%	*Gram*	*Gram*	*Gram*	*Gram*	*Gram*	*Gram*
80	0.0200	0.0201	0.0001	0.0215	0.0219	0.0004
85	0.0200	0.0201	0.0001	0.0215	0.0219	0.0004
90	0.0199	0.0201	0.0002	0.0216	0.0219	0.0003
95	0.0201	0.0201	0.0000	0.0216	0.0219	0.0003

Discussion

Table I is further confirmation of the findings of Ford and Hughes that higher percentages of ethanol give higher potash values.

Table II indicates that, within experimental error, the composition of the potassium chloroplatinate precipitate is not changed by varying the concentration of the ethanol used.

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Determination of Aluminum in Biological Material

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THE application of a spectrochemical method to the determination of aluminum in biological material (*5*) yielded results which were often lower than those previously reported by many other workers (*14*). A careful study of the method resulted not only in a number of modifications which greatly increased the precision of analysis in the lower range of concentrations but also in the development of a chemical method which could be used to check the spectrographic findings. The refinements incorporated in the spectrochemical method and the details of the chemical method employed in testing it are described in the following paragraphs.

Spectrochemical Method

The chief difficulty in the use of the spectrochemical method was encountered in its application to extremely low levels of concentration where precision is affected by background fog and residual aluminum in the reagents and electrodes. The residual aluminum even in the case of individual electrodes may vary considerably and therefore a blank correction cannot be used with any degree of safety. While a number of earlier batches of electrodes were satisfactorily purified by means of prolonged acid extraction (*6*), this treatment has not proved consistently reliable for more recently purchased batches. This fact led to an examination of available purified electrodes and to the choice of 0.6-cm. (0.25-inch) Dow purified graphite electrodes for this work.

The reagents to be employed are purified either by redistillation (distilled water, nitric and hydrochloric acids) or, when possible, by the removal of aluminum as the phosphate (at pH 4.2). The latter method has proved satisfactory for the purification of the spectroscopic base (*4*) which is used to derive the calibration curves and as the diluent added to the prepared samples to control their inorganic salt composition (*4*). The small quantities of residual aluminum which, together with plate emulsion fog, affect the reliability of the lower sections of the working curves may be determined spectrographically when the working curves are derived (*7*).

The reliable evaluation of photometric data obtained for lines, the intensities of which are just above that of the emulsion fog, has lately been the subject of a number of papers (*7, 15, 17, 20*). The method used to correct for background fog in this study is fundamentally the same as that described by Pierce and Nachtrieb (*17*), with the additional precaution that the internal standard employed is a line in the same range of intensity as the test line (*7*). The advantage of such a procedure is obvious from the fact that the values for the density or intensity of lines in the medium and upper blackening levels suffer little change due to background. With 15 mg. of bismuth per 100 ml. of prepared test solution (0.2 ml. of which are placed on the arc) the bismuth lines at 2993.4 and 3024 are of suitable intensity to serve as standard lines for the evaluation of aluminum (3082 Å.) in the respective ranges of from 0.01 to 0.10 mg. per 100 ml., and from 0.05 to 2.00 mg. per 100 ml., of the prepared sample.

The nitric acid solutions (plus hydrochloric acid, if tin is present) of the dry-ashed (500° C.) samples are adjusted to volumes dictated by experience. Generally the solutions of ashed solid tissues, blood, and individual food items are adjusted to volumes corresponding to the weight in grams of the original material. The solutions of ashed urine samples are made up so that each milliliter corresponds to 10 ml. of fresh urine, those of feces ash to a total volume of 100 ml., and the solutions of ashed mixed food samples to 500 ml.

In the case of urine the internal standard (1 ml. of a solution containing 1.5 mg. of bismuth) is included in making the final volume adjustment. Other materials are prepared for spectrographic analysis by mixing—in graduated 15-ml. Pyrex centrifuge tubes—suitable quantities of the prepared sample solutions and definite amounts of a spectroscopic buffer salt solution containing the proper amount of the internal standard (15 mg. of bismuth per 100 ml. of buffer solution). (The buffer solution or diluent contains the inorganic salts of ashed urine and is adjusted in volume so that 1 ml. is equivalent to 10 ml. of normal urine, *4*.) The mixtures are concentrated in a glycerol or oil bath to the volumes of diluent employed, and 0.2-ml. portions are dried in the craters of graphite rods (3.75 cm., 1.5 inch, in length and 0.6 cm., 0.25 inch, in diameter). The dried rods are then burned (with similar untreated rods serving as negative electrodes) for 2 minutes as a direct current arc from a 120-volt line at 10 amperes and with a 50-volt drop across the electrodes. The craters (10 mm. in depth and 3 mm. in diameter) in the two electrodes help to improve the steadiness of the arc. Step spectra are obtained by rotating a 5-step sector (factor of 2) before the slit of a Bausch & Lomb large quartz Littrow spectrograph (set at 5) and photographing the refracted light on Eastman No. 33 plates. The step densities for the test and standard lines are measured in a nonrecording densitometer and the separation between the respective Hurter-Driffield curves (plotted as densities against the log relative exposures of the steps) at a constant density (0.30) gives the aluminum concentration when read from a calibration curve derived by adding known amounts of aluminum to the spectroscopic buffer (*6*). In the case of very weak lines (0.01 to 0.10 mg. of aluminum per 100 ml. of solution), only the maximum exposure steps are used, as described in a recent paper (*7*). In this case it is advisable to correct for background fog, and with urine samples to correct as well for any residual aluminum which may have been present in the salt stock used to derive the working curve (Figure 1 and *7*).

Table I. Aluminum Recovered by Chemical Method

(Using 200 mg. of diammonium phosphate for precipitation)

Range Used	Al Added	Al Found	Al Recovered
Micrograms	*Micrograms*	*Micrograms*	*Micrograms*
0–5	0	5.0	..
0–5	0	5.2	..
0–5	0	4.9	..
		Av. 5.0	
0–5	1	6.3	1.3
0–5	1	5.9	0.9
0–5	1	6.1	1.1
0–50	5	10	5
0–50	5	12	7
0–50	5	11	6
0–50	30	35	30
0–50	30	37	32
0–50	30	35	30
0–50	50	57	52
0–50	50	54	49
0–50	50	55	50

Chemical Method

None of the available chemical methods was adequate for the authors' purpose, since they needed a method which could be applied over a wide range of values (1 microgram to several hundred milligrams).

Schmidt and Hoagland's aluminum phosphate method (*19*) is excellent for gravimetric work but obviously could not be used for minute quantities. The available micromethods employing 8-hydroxyquinoline (oxine), the ammonium salt of aurin tricarboxylic acid (aluminon), or Alizarin Red S, were not suitable because of losses and manipulative difficulties.

The oxine methods as described by Berg (*3*), Hahn and coworkers (*10, 11*), and Schams (*18*) were time-consuming and inadequate for amounts less than 50 micrograms of aluminum. Quantities below this value did not precipitate readily as the oxinate, a fact which Alten and co-workers had reported (*1*). In addition, the many manipulative steps entailed unavoidable mechanical losses. The color obtained by coupling the diazotized oxine (liberated from the aluminum oxinate) with sulfanilamide was photometrically suitable, however, and therefore the method could readily be applied to quantities of aluminum above 50 micrograms.

The aluminon method has been employed by a number of investigators (*9, 12, 16, 22–25*). That described by Cox (*9*) was chosen as the best representative of these methods, but was found to be unsatisfactory. The principal difficulties encountered were loss of aluminum through adsorption or occlusion when interfering iron was removed as the hydroxide, interference by the phosphate ion in the final estimation, and failure of the color to obey Beer's law.

The Alizarin Red S method, first described by Atack (*2*), then carefully studied by Yoe and Hill (*26*) and applied by Underhill and Peterman (*21*), appeared to be the method best suited to the authors' purpose. With certain modifications which are included in the description below, it has the following advantages over others using Alizarin Red S: The isolation of aluminum as the phosphate followed by washing with water (and the subsequent removal of iron with cupferron) removes interfering substances which affect the final color (*2*); employment of a mixed color makes it possible to avoid the use of acetic acid, which attacks the aluminum lake (*2*); and this colorimetric procedure permits the use of a single stable calibration curve which obeys Beer's law.

Method of Procedure

Isolation of Aluminum as Aluminum Phosphate. Samples of urine (100 ml. or less), blood (5 grams or less), tissues (5 to 100 grams), feces (24-hour excretion), and mixed food are prepared for analysis by the dry-ashing method used for spectrographic work (*4*).

Transfer the entire prepared sample of urine or blood—or a suitable aliquot in case the aluminum content is greater than 50 micrograms—to a 50-ml. graduated conical centrifuge tube. Add 1 mg. of iron (100 mg. of iron as ferric chloride per 100 ml. of water), 1 ml. of saturated ammonium acetate solution, and 200 mg. of diammonium phosphate (4 grams of diammonium phosphate per 100 ml.). Dilute to 20 ml., mix well, and add 6 drops of 0.1 per cent aqueous bromocresol green solution. making certain that all phosphates are in solution by adding, if necessary, a few drops of 6 *N* hydrochloric acid. Add dilute ammonium hydroxide (30 ml. of concentrated ammonium hydroxide per 100 ml.) drop by drop until pH 4.2 is obtained (by matching against a standard buffer, *8*).

Dilute to 30 ml. and heat tube and contents in a bath of gently boiling water for approximately 30 minutes. Wash down sides of tube with a fine jet of hot water, bring the volume to 30 ml., and centrifuge for 10 minutes at 1800 r. p. m., discarding supernatant liquid. Wash down the sides of the tube with about 2 ml. of hot water, loosen the precipitate containing aluminum phosphate plus iron phosphate, breaking up hard lumps with the pointed end of a glass stirring rod, and dilute to approximately 20 ml. Coagulate the phosphate by placing the centrifuge tube in a water bath for 10 minutes; then centrifuge again for 10 minutes, discarding the supernatant liquid. It is not necessary to add diammonium phosphate to prepared urine samples or iron to prepared blood samples. Moreover, in the case of blood the amount of phosphate normally present permits satisfactory precipitation of aluminum, if only 100 mg. of the diammonium phosphate are added.

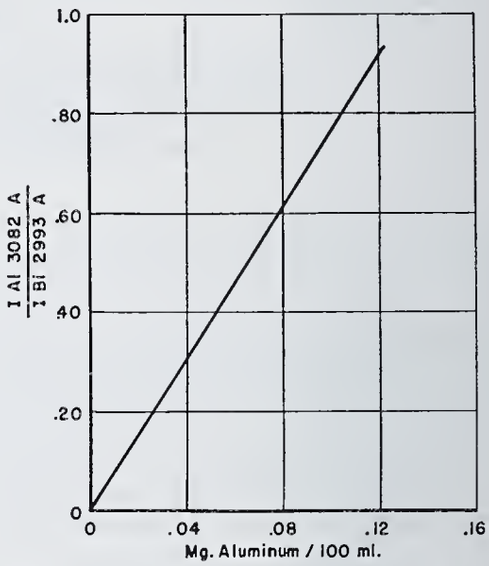

Figure 1. Calibration Curve for Spectrographic Determination of Micro Quantities of Aluminum

Corrected for background and residual aluminum

Removal of Iron Interference. Dissolve the precipitate with 5 ml. of dilute sulfuric acid (10 ml. of concentrated sulfuric acid per 100 ml.), place in the hot-water bath to effect solution, dilute to approximately 10 ml., and centrifuge for 10 minutes to separate silica. Transfer the supernatant liquid to a 150-ml. Squibb type separatory funnel and dilute to 20 ml. Add 2 ml. of a cold aqueous cupferron solution (6 grams per 100 ml.) and allow to stand for 1 minute after thorough shaking. Add 10 ml. of a water-saturated benzene-ether mixture (equal parts by volume), shake for 1 minute, and allow complete separation of the two phases. Remove the aqueous layer to a second separatory funnel and repeat treatment with 10 ml. of the benzene-ether mixture. Remove the aqueous fraction to a 30-ml. Kjeldahl flask containing 1 ml. of concentrated nitric acid, add one or two small clean Carborundum bumping stones, and heat with a microburner until fumes of sulfur trioxide appear. Cool, add 1 ml. of water, and again heat until appearance of sulfur trioxide fumes, to expel oxides of nitrogen.

Photometric Measurement of the Mixed Color of the Aluminum Lake of Alizarin Red S Plus Excess Alizarin Red S. If the range of 0 to 5 micrograms of aluminum is to be used, rinse the contents of the 30-ml. Kjeldahl flask completely into a 25-ml. glass-stoppered cylinder. Dilute to 14 ml., add 1 ml. of an aqueous solution of Alizarin Red S (0.075 gram of Alizarin Red S per 100 ml.), then add 10 ml. of dilute ammonium hydroxide (30 ml. of concentrated ammonium hydroxide per 100 ml.), mix the contents, and measure the density immediately at 580 mμ, with a suitable spectrophotometer.

If the range is 0 to 50 micrograms of aluminum, use a 100-ml. glass-stoppered cylinder. Dilute to 85 ml., and add 5 ml. of

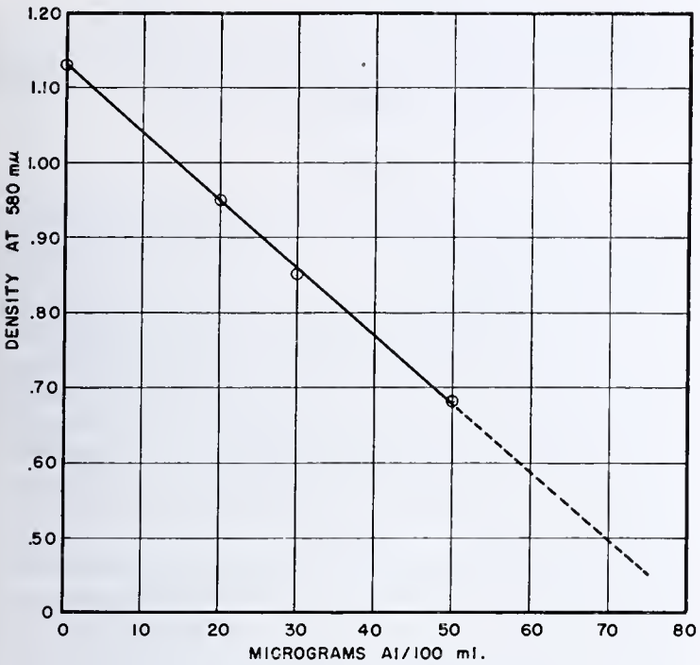

Figure 2. Calibration Curve for Spectrophotometric Measurement of Mixed Color

Cell length 2.5 cm.

the same Alizarin Red S solution and 10 ml. of dilute ammonium hydroxide. Other procedures are the same.

The density curve as shown in Figure 2 for the range 0 to 50 micrograms was obtained with a pure aqueous solution of aluminum chloride, containing 5 ml. of dilute sulfuric acid (10 ml. of concentrated sulfuric acid per 100 ml.). Density measurements were obtained with the photoelectric spectrophotometer described in a previous paper (*13*). A reagent blank of approximately 5 micrograms of aluminum is obtained when 200 mg. of diammonium phosphate are used.

Results

Table I lists the recoveries obtained by the chemical method when known amounts of aluminum were added and 200 mg. of diammonium phosphate were used for precipitation of aluminum. The agreement between findings obtained by the chemical and the spectrographic methods is shown in Tables II, III, and IV. Table II gives recoveries of known amounts of added aluminum approximating those which may be encountered in fecal and mixed food samples. Table III illustrates the agreement between the results obtained by the two methods at the lower levels of concentration. These results, listed in the frequencies of their occurrence, with their calculated mean values, were obtained from duplicate samples of consecutive daily urinary specimens from a subject under study. The range of application of the two methods, the agreement in the findings obtained by their use, and also the levels of aluminum concentration which may be encountered in miscellaneous material may be seen in Table IV.

Discussion

Aluminum is the most widely distributed of the metals, forming as it does about 7 per cent of the earth's surface. Contamination, therefore, is a serious factor in the determination of aluminum and its complete avoidance is practically impossible. Nevertheless, the consistently low results and the close agreement between the means obtained on independent aliquots of urine samples by two such distinctly different methods (Table III) point to the fact that contamination has been held to a minimum. The effect of adventitious aluminum depends, of course, on the range of concentrations

Table II. Comparison of Analytical Results Obtained by Spectrographic and Chemical Methods

Feces Sample	Al Added	Chemical Method: Al found	Chemical Method: Al recovered	Spectrographic Method: Al found	Spectrographic Method: Al recovered
	Mg.	*Mg.*	*Mg.*	*Mg.*	*Mg.*
0721	0	1.66	..	1.84	..
	0	1.66	..	1.80	..
	0	1.40	..	1.64	..
	Av.	1.57	Av.	1.76	
0721	5	6.66	5.09	6.50	4.74
	5	6.50	4.93	7.25	5.49
	5	6.16	4.59	6.75	4.99
		Av.	4.87	Av.	5.07
0721	10	12.00	10.43	11.20	9.44
	10	12.25	10.68	11.30	9.54
	10	10.75	9.18	12.50	10.74
		Av.	10.07	Av.	9.90
0721	100	105.0	103.4	114.8	113.0
	100	105.0	103.4	109.8	108.0
	100	92.5	90.9	101.8	100.0
		Av.	99.2	Av.	107.0

Table III. Determination of Aluminum in Consecutive Samples of Urine from an Experimental Subject

(Comparison of spectrographic and chemical results)

Al Found, Mg./l.	Frequencies of Occurrence: Chemical	Frequencies of Occurrence: Spectrographic
0.010–0.0149	1	..
0.015–0.0199	2	..
0.020–0.0249	7	..
0.025–0.0299	4	..
0.030–0.0349	7	4
0.035–0.0399	13	8
0.040–0.0449	9	25
0.045–0.0499	12	10
0.050–0.0549	13	23
0.055–0.0599	6	4
0.060–0.0649	4	9
0.065–0.0699	4	3
0.070–0.0749	5	2
0.075–0.0799	0	0
0.080–0.0849	4	3
Totals	91	91
Mean	0.046	0.050
Probable error	±0.001	±0.001
Std. deviation	±0.016	±0.011
Coefficient of variation	34.59%	21.75%

encountered and may be serious in the case of urine, blood, or animal tissues with a low aluminum content. It is therefore advisable to carry out analyses in duplicate whenever sufficient material is available.

Silica does not interfere with the determination and there is no evidence that any considerable portion of the aluminum present in prepared samples of feces or mixed food is in the form of insoluble silicate. A number of tests on such material containing 1.5 to 100 mg. of aluminum showed that 99 per cent or more of the aluminum was in the soluble form. The fact that in one case 1 per cent of the aluminum was found in the silica does not mean that the aluminum was present as an insoluble silicate; a more probable explanation is that it was difficult to remove small amounts of adsorbed aluminum completely by washing. Silica is removed in the chemical method for photometric reasons only.

At least 1 mg. of iron was found to be essential for the complete adsorption or entrainment of 50 to 75 micrograms of aluminum as the phosphate. Higher amounts are unnecessary, and were avoided because of the increased manipulation required in washing the phosphate and in removing the iron with cupferron. Phosphate must be present in an amount in excess of that needed to combine with all of the added iron and the aluminum. Best results were obtained when 200 mg. of diammonium phosphate were employed. Larger amounts give rise to high blanks, and, in addition, increased quantities lead to incomplete removal of excess diammonium phosphate by the washing process. The phosphate remaining, when carried through to the estimation step, may cause a partial precipitation of aluminum phosphate and thus produce low

Table IV. Comparison of Analytical Results Obtained by Spectrographic and Chemical Methods

Sample	Aluminum Found Spectrographic	Aluminum Found Chemical
	Mg./l.	*Mg./l.*
Urine 803	0.04	0.03
Urine 1483	0.06	0.06
Urine 1495	0.08	0.07
	Mg./100 g.	*Mg./100 g.*
Blood 606	0.02	0.03
Blood 843	0.03	0.03
Blood 945	0.03	0.03
	Mg./24 hours	*Mg./24 hours*
Feces 3496[a]	338	332
Feces 4188	12.5	12.6
Food 3145[a]	330	324
Food 3717	3.6	3.9

[a] Following addition of Al to diet.

results. This fact also limits the upper range of estimation by chemical means to 50 or 75 micrograms per aliquot, since larger quantities of aluminum also tend to recombine with the phosphate under the conditions of this method.

The only difficulty encountered with the chemical method as outlined in this paper is in establishing the range of the concentration of aluminum in the specific sample; this can be done only on the basis of experience with a particular series of samples. In the case of urine and blood samples, the aliquots designated usually can be analyzed with the 0 to 5-microgram curve, with extrapolation to 7.5 micrograms if necessary. This procedure is preferable to using a 0 to 10 microgram range, since the quantities of alizarin required in this case give an initially high density and thus make it difficult to evaluate small color changes. However, since the method is sensitive, small aliquots can be used, and the analysis can be repeated with larger aliquots when this is deemed advisable.

The stability of the lake formed is sufficient for rapid measurement—within 10 to 15 minutes. Under the conditions outlined the densities of the mixed color (with increased aluminum) follow Beer's law (Figure 2), the greatest density spread between the aluminum-lake color and the excess Alizarin Red S color being observed at 580 mμ. For most accurate results each sample should be developed immediately before reading.

With the spectrographic method there is no question of specificity when an instrument of sufficient dispersion (large quartz Littrow type) is employed. The problem here, as previously pointed out, is one of reproducibility, particularly for the extremely low concentration ranges. The reproducibility obtainable by the present method is excellent when judged by the variations in individual results used to plot Figure 1. A varying number of spectra was employed for each point. Table V shows number of spectra and mean error of a single analysis as read from the finally derived curve, plotted from the mean values at a number of concentrations.

In the higher concentrations the average error of spectrochemical analysis is approximately ± 10 per cent. The evaluation of spectrum-line blackenings by the usual interval of log E separation (*6*, *7*) may be used with excellent results for quantities above 0.05 mg. of aluminum per 100 ml. of solution. The older method of evaluating weak spectrum lines by the method of opacity separation (*6*, *7*) may also be used when background is present, provided the standards used to derive the curve had approximately the same intensity of background. This older method fails particularly in dealing with very weak lines in spectra having practically no background. In the latter case, the separations are too large, and consequently they yield low results. Although the bismuth 3024 line has been used as an internal standard it is also possible to use the 2898 line (*7*). When this is done the amount of bismuth added should be reduced to 5 mg. per 100 ml. Under these conditions the 2993.4 line used for the evaluation of weak lines will be reduced in intensity to a level corresponding to that obtained with the aluminum 3082 Å. line, when the range is from 0.01 to 0.05 mg. of aluminum per 100 ml.

Summary

A spectrochemical method and a chemical method are described for the determination of aluminum in biological material in the range of from 1 microgram to several hundred milligrams per sample.

The precision of analysis of quantities of aluminum in the range of 0.01 to 0.10 mg. per 100 ml. of solution by spectrochemical means has been improved by correcting for background fog. The method of background correction found most satisfactory entails the use of standard lines of the same degree of intensity as that of the aluminum line in the low concentration range. The average error of a single analysis in the low range is ± 0.014 mg. of aluminum per 100 ml. of solution.

The chemical method, which equals the spectrochemical method in precision, consists of the isolation of aluminum as the phosphate, the removal of iron with cupferron, the subsequent development of an aluminum lake with Alizarin Red S, and the measurement of the mixed color by means of a photoelectric spectrophotometer.

Table V. Reproducibility of Findings by Spectrographic Means

(Low concentration range)

Concentration *Mg./100 ml.*	Spectra	Mean Error of Single Analysis *Mg./100 ml.*
0.03	6	± 0.012
0.04	6	± 0.010
0.08	11	± 0.015
0.13	10	± 0.019

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Barcroft-Warburg Manometric Apparatus

Usage, Recent Developments, and Applications

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WHEN reference is made to the use of the Barcroft-Warburg respirometer in experimental methods it is usually understood that the apparatus consists of a manometer fitted with a removable sample vessel or flask, and a suitable constant-temperature water bath equipped with an efficient thermoregulator, stirrer, and shaking mechanism. There are essentially three standard types of manometers ordinarily used with this equipment. The first, and most common type, is referred to as the Warburg or constant-volume respirometer, so named on account of its extensive use by O. Warburg and his colleagues at Berlin, although it is similar in design and construction to the original "blood gas manometer" employed by Barcroft and Haldane (*6*) in 1902.

Constant-Volume Respirometer

The Warburg manometer, as illustrated in Figure 1, consists of a Pyrex U-tube with an outside diameter of 7 to 8 mm., carefully selected for uniformity and precision of bore. For proper sensitivity, the internal cross section of the bore should be approximately 1 sq. mm. The vertical arms of the tube are accurately graduated in millimeters from 0 to 30 cm., and the horizontal alignment of the graduations is of the order of 0.1 mm. At the base of the manometer a rubber reservoir and screw-clamp arrangement are attached, by which the level of the indicating liquid in the tube may be adjusted. One end of the U-tube is open to the air and the other end, as shown, is constructed with a horizontal arm for attaching a suitable vessel by means of a standard taper (Ŧ) interchangeable ground-glass joint. A three-way stopcock not only permits communication with the air but also serves for the introduction of different gas mixtures into the respiring material, dependent upon the experimental requirements.

Each manometer of this type is equipped with a removable support or backing made of Monel metal or wood and also a special sleeve for attaching to the shaking mechanism of the thermostat as described below. Such an arrangement has been found necessary in view of the fact that when the manometer is in its proper position the sample vessel must be completely immersed in the constant-temperature water bath.

Before making a reading on the Warburg type of manometer, the level of the indicating fluid in that arm of the U-tube connecting with the test vessel is always adjusted by means of the thumbscrew device to the same given point—for example, the 150-mm. mark. Readings are made on that arm of the manometer which is open to the air only, and the difference of pressure between the interior of the vessel and the outer air is thus obtained by subtracting 150 from these readings. From the reading of the manometer the volumes of gas absorbed or evolved by the respiring material can be calculated according to the following formula:

$$x = h\left[\frac{V_g \frac{273}{T} + V_f(\alpha)}{P_0}\right] \qquad (1)$$

where

x = cu. mm. of gas at standard temperature and pressure

h = reading of manometer (change in height in manometric graduations)

V_g = free volume of gas in the vessel and manometer to the level of manometric fluid (total volume of apparatus less volume of sample, liquids, and detachable supports placed in the vessel)

T = absolute temperature of the water bath surrounding the vessel

V_f = volume of all liquids in the vessel in which the measured gas might dissolve

α = solubility of the gas being measured in the vessel liquid at temperature T (see Bunsen's solubility table)

P_0 = normal pressure in terms of manometric fluid (for Brodie's solution, 10,000 mm.). Brodie's solution may be prepared as follows: sodium chloride, 23 grams; sodium tauroglycocholate, 5 grams; water to 500 ml. (10,000 mm. of this solution are approximately equivalent to 760 mm. of mercury).

In experiments where a constant volume of material is to be used, the quantity within the brackets in Formula 1 remains constant for a given vessel and is referred to as the vessel constant, k. The majority of vessels used with the Warburg respirometer possess volumes of approximately 15 ml. and their respective constants usually range from 1 to 3. Therefore, if the vessel constant is known, it is only necessary to multiply the manometer reading by it to find the amount of gas evolved at standard temperature and pressure.

MacLeod and Summerson (*55*) have recently described a graphic method for determining Warburg vessel constants at various fluid volumes. This type of procedure eliminates the tedious computations and is, no doubt, conducive to greater accuracy, since a linear relationship exists between the vessel

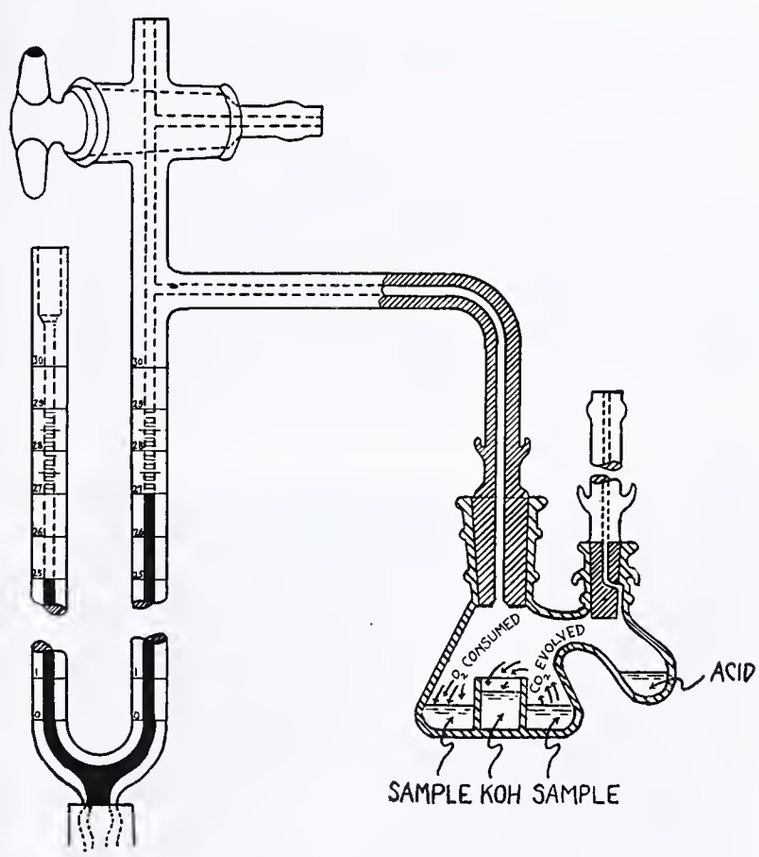

FIGURE 1. WARBURG CONSTANT-VOLUME MANOMETER AND REACTION VESSEL

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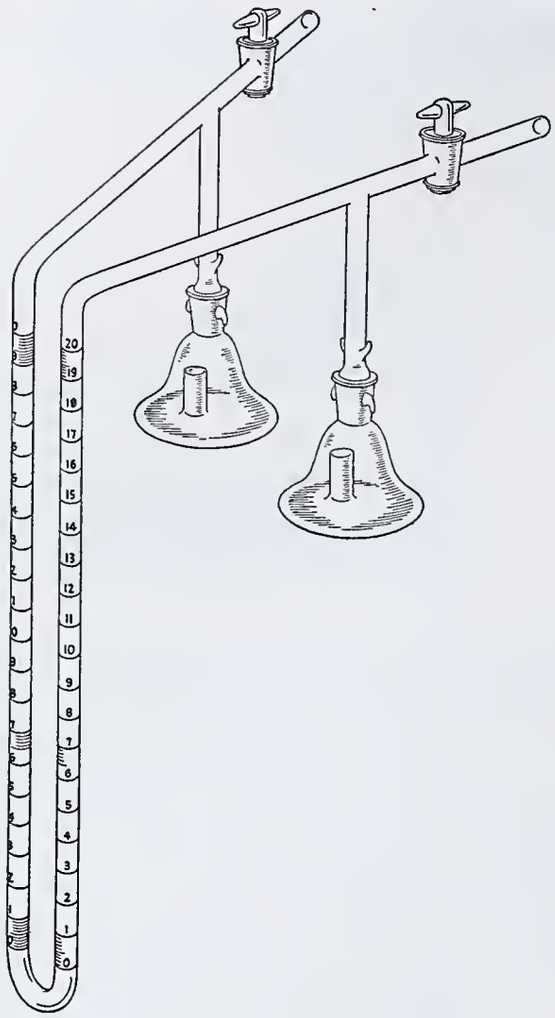

FIGURE 2. BARCROFT DIFFERENTIAL MANOMETER WITH FLASKS

constant, k, and the volume of liquid in the vessel under ordinary conditions.

Barcroft Differential Respirometer

The second most popular type of instrument used in microrespiration studies is, perhaps, the differential respirometer developed by Barcroft (*5*) in 1908. It is similar in construction to that used earlier by Warburg (*91*) in measurements of the rapidity of ozonization. The differential manometer differs principally from the Warburg type by having one end not open to the air but connected to a second flask which, in turn, acts as a compensating vessel and thus eliminates errors due to slight changes of temperature or barometric pressure during the experiment.

An illustration of the Barcroft respirometer is shown in Figure 2. This is the form now generally employed and particularly recommended by Dixon and Elliott (*22*) for respiration studies and similar processes, in which the rate of the process rather than the total amount of gas absorbed is to be determined. No screw adjustment is required in this type of manometer, as the indicating liquid is allowed to move freely in both arms of the U-tube and the readings are made by simply noting the difference between the readings of the two arms. The U-tube is constructed of Pyrex tubing with an outside diameter of 6 to 7 mm. and a bore of approximately 2 sq. mm. with graduations from 0 to 200 mm. (*21*). It is essential that this style of manometer be provided with two special flasks whose volumes are exactly equal to within 0.1 ml.; otherwise, the compensation will not be perfect and serious errors may be introduced.

The type recommended by Dixon and Keilin (*23*) with a capacity of about 40 ml., has been found very satisfactory, since it is made with a removable side arm and hollow stopcock for introducing acid or alkali during an experiment. This style of flask is shown in Figure 3. In this type of manometer also it is necessary to determine the apparatus constant, k, which may be derived from the following simplified formula:

$$x = h\left(\frac{A}{2}\frac{P_0}{V_g'}\right)\left(\frac{V_g\frac{273}{T} + V_f\,\alpha}{P_0} + \frac{A}{2}\frac{273}{T}\right) \qquad (2)$$

where

x = cu. mm. of gas evolved in reaction vessel (right flask)
h = reading of manometer
A = area of bore in manometer (sq. mm.)
V_g' = volume of gas space in compensating (left) flask, including manometer tubes to the level of manometric fluid, normally kept at 100-mm. graduation
V_g = volume of gas space in reaction vessel (right flask)
T = absolute temperature of water bath
α = solubility of gas being measured in vessel liquid at temperature T
V_f = volume of all liquids in the vessel in which the measured gas might dissolve
P_0 = normal pressure in terms of manometric fluid

The Barcroft respirometer as modified by Dixon and Elliott (*22*) is preferred by a great many workers for measurements involving the oxygen uptake, the respiratory carbon dioxide production, and glycolysis of tissue slices or homogenized tissue suspensions (*26*, *62*, *77*, *102*).

Summerson Manometer

The third style of manometer in current use with the Barcroft-Warburg apparatus is an ingenious device recently developed by Summerson (*83*) and illustrated in Figure 4. It offers in a single apparatus the possibilities of either direct or differential manometric measurements. For direct measurements it is the equivalent of two single manometers of the conventional Warburg type in which one arm of the manometer is open to the atmosphere. When used as a differential manometer it operates at constant volume, with the attendant simplification of differential measurement made possible by use of constant-volume vessel constants.

Readings on the Summerson manometer are made along the two inner arms which are graduated from 0 to 450 mm. The manometer fluid levels and vessel volumes are maintained constant at the calibration marks on the outer arms by the usual method of adjusting the pressure screw device on the fluid reservoir. The differential readings are equally precise at any vessel pressure, and a sensitive manometer fluid may be used for the determination of small differences in pressure in the presence of relatively large total pressures. This type of manometer is adaptable to a number of different manometric procedures, each of which has heretofore required a separate type of instrument. It is particularly suited to the differential measurement of the oxygen consumption, respiratory carbon dioxide output, and aerobic glycolysis in metabolism studies.

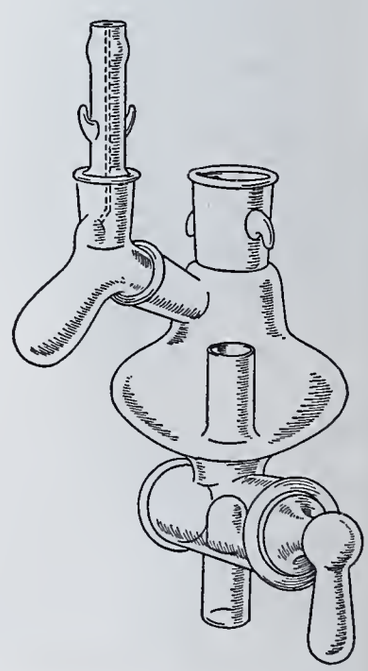

FIGURE 3. DIXON-KEILIN FLASK

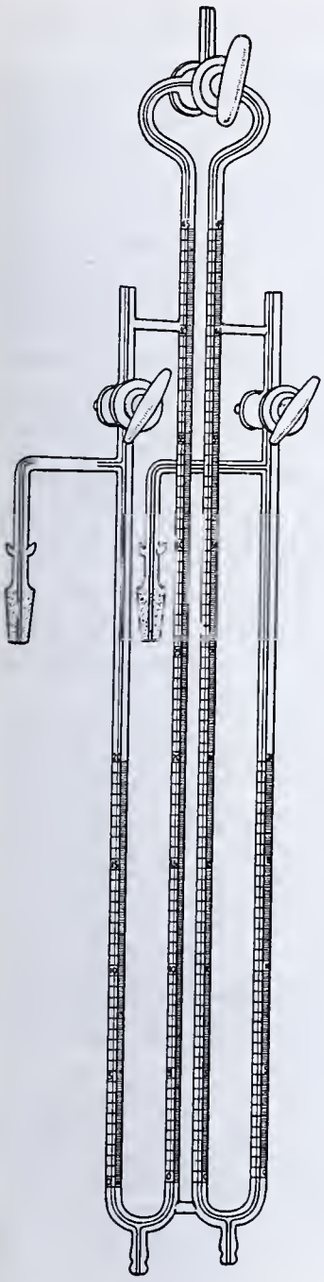

FIGURE 4. SUMMERSON COMBINATION MANOMETER

Constant-Temperature Bath

Close and reliable temperature control is of the utmost importance in the Barcroft-Warburg procedures, since the apparatus is arranged to measure kinetically the oxygen consumption as a function of time under conditions of constant temperature and atmospheric pressure. Precise temperature control of the bath or thermostat to ±0.02° C. or better is accomplished by means of an efficient metastatic mercury or bimetal thermoregulator, a sensitive relay, motor stirrer, and an immersion heater arranged to provide a symmetrical distribution of heat throughout the bath.

As shown in Figure 5, the shaking mechanism operating on ball-bearing rollers is an integral part of the assembly and permits the attached manometers to move through the bath with a horizontal reciprocating motion, thus allowing readings to be made without stopping the shaking action. By means of a rheostat control various speeds of shaking up to about 200 complete oscillations per minute with an adjustable amplitude of movement from 0 to 4 cm. can be obtained, and, providing the shaking mechanism is driven by a synchronous motor, any speed selected will be constant regardless of variations in load or line voltage. Dixon and Elliott (*22*) demonstrated that the rate of shaking is a very important factor, especially when studying the velocity of oxygen uptake of materials such as baker's yeast.

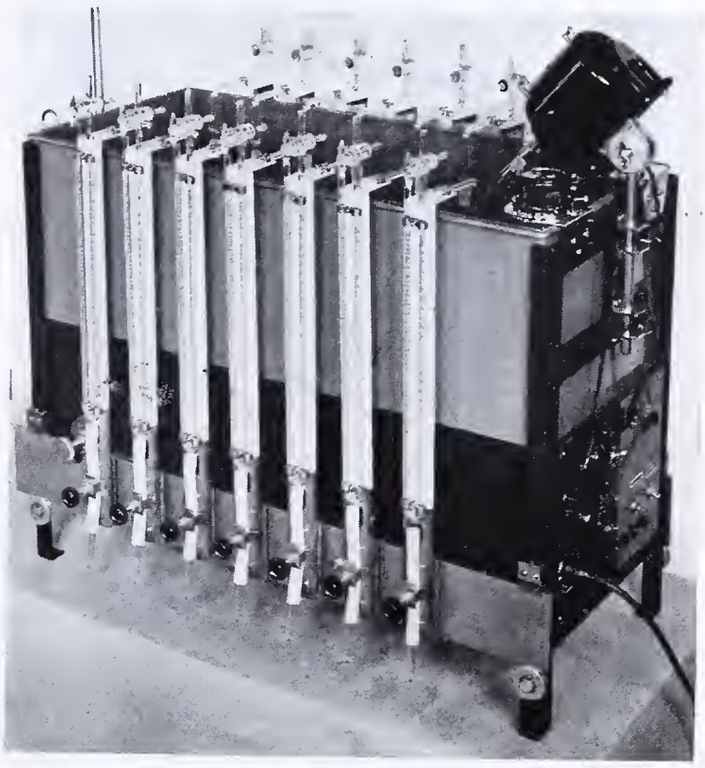

FIGURE 5. BARCROFT-WARBURG APPARATUS EQUIPPED WITH 14 MANOMETERS

Recent Developments and Modifications

One of the most widely used and versatile modifications of the Barcroft-Warburg apparatus is the differential volumeter devised independently by Fenn in 1927 (*28*) for the measurement of cellular respiration and other processes. This highly sensitive instrument is illustrated in Figure 6. It operates upon the volumetric rather than the manometric principle and is similar in construction to the differential volumeter first introduced by Thunberg in 1905 (*87*), and later modified by Winterstein (*99*) and Widmark (*98*).

The instrument consists essentially of a control flask and an experimental flask connected by a capillary tube containing an index drop of kerosene stained with Sudan III dye to improve its visibility. The capillary is constructed of precision-bore Pyrex tubing with an internal cross section of approximately 0.3 sq. mm. (to contain 3 cu. mm. per cm. of length), and graduations extending from 0 to 15 cm. in 1-mm. subdivisions. The experimental flask has two side arms situated 90° apart to facilitate the transfer of contents separately, and also a stopcock for the introduction of different gas mixtures if needed. Sodium hydroxide may be placed in one side arm to absorb carbon dioxide and the other may contain any reagent which it is desired to introduce during the experiment. The volume of each flask is approximately 15 ml., although flasks possessing smaller volumes can be used with a resulting increase in sensitivity. This instrument also requires the use of a constant-temperature water bath similar to the one described above.

As oxygen is consumed in the experimental flask the index drop is drawn along the capillary tube, the carbon dioxide being absorbed in the alkali. If the gas spaces in both flasks are equal, the loss of gas due to absorption in the one is shared equally by the other in order to keep the pressure equal on both sides of the index drop. The drop, therefore, moves through a volume of capillary equivalent to one half the volume of the gas absorbed.

Fenn (*29*) has clearly presented the theoretical principles governing volumeter measurements and further description is not necessary. However, with the differential volumeter a greater sensitivity can be obtained (to 0.001 μl.) without reducing the volume of the vessels to inconvenient sizes. It

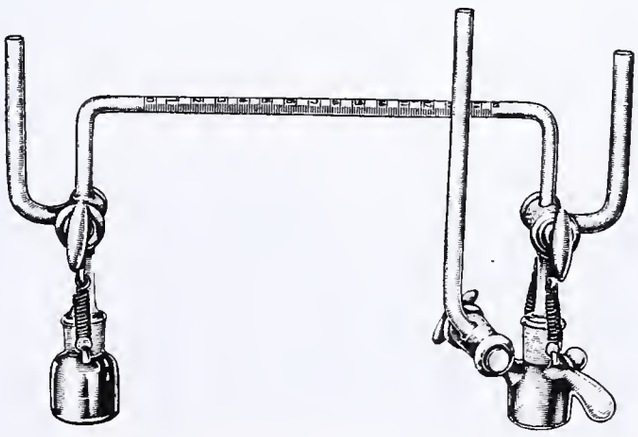

FIGURE 6. FENN DIFFERENTIAL VOLUMETER

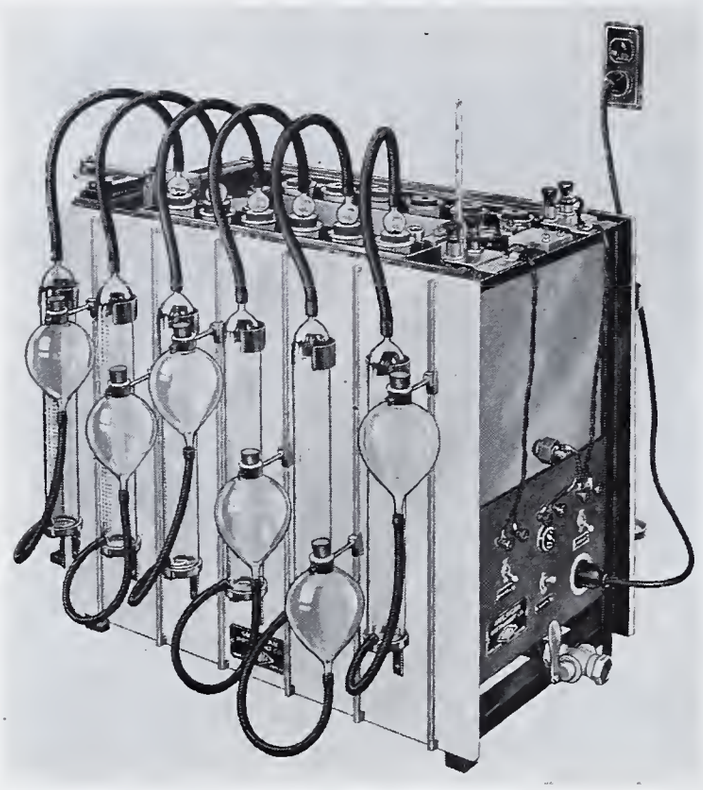

FIGURE 7. FLEISCHMANN-AMINCO FERMENTOMETER

is simple in operation, less expensive, more compact, and can be depended upon to yield results equal to the manometric methods in convenience and precision.

The subject of volumetric versus manometric methods for the determination of gas production has been discussed thoroughly by Eisenberg (*25*). Jares (*41*), Martin (*56*), and Burkholder (*16*) have used the differential volumeter for determinations of the respiratory quotient and oxygen consumption of certain bacteria and tissues. Recently Goodwin and Goddard (*34*) were highly successful in using the Fenn apparatus for measuring accurately the gaseous changes which take place between isolated woody tissue sections and their environment. They found the apparatus to be sensitive to 0.15 cu. mm. of gas per hour at 25° C. Schmitt (*69*) modified the differential volumeter for still higher sensitivity and applied it to the determination of the oxygen consumption of stimulated nerve. Gerard and Hartline (*32*) modified this instrument by taking advantage of the greater stability afforded by reducing the tissue chamber to capillary dimensions (0.5 to 1.2 mm. diameter) and placing this inside a relatively large "differential" chamber. Index drop movements, observed with an ocular micrometer, were consistent over 5-minute intervals, with volume changes of the order of 0.01 cu. mm.

A further development of the capillary method has recently been made by Tobias and Gerard (*88*), so that it is now convenient to follow the respiration of ten tissue samples at once, and it is possible to measure absolute gas volume changes of 0.001 cu. mm., minute by minute, with an error of some 8 per cent minute by minute; for longer intervals the error is less than 1 per cent. With the capillary microrespirometer the reliability of QO_2 values is limited by the accuracy of estimation of the amount of respiring material. (QO_2 is the symbol for respiration rate and may be defined as the number of cubic milliliters of oxygen absorbed per hour per milligram of dry might of tissue at standard temperature and pressure.)

Another form of microrespirometer operating upon the differential volumeter principle has been developed by Thimann and Commoner (*86*). It permits volumetric readings to be made at 1-minute intervals and is very sensitive to small or transient changes in respiration rate (a drop movement of 1 mm. indicates a volume change of about 0.06 cu. mm.). It is capable of general use with small amounts of biological materials, tissues, or organisms, and provides for convenience in manipulation.

Asymmetrical differential respirometers have been constructed by Duryee (*24*) and by Victor (*90*), with somewhat lower sensitivities (0.1 and 0.01 μl., respectively). Stefanelli (*82*) has described an instrument similar to that of Victor, with a sensitivity of 0.003 μl. for measuring the effect of cleavage on the respiration of a single egg of *Rana fusca*. Laser (*52*) and Meier (*57*) used a modified Warburg respirometer with a sensitivity of 0.1 μl. for the measurement of the respiration of tissue cultures of *Drosophila melanogaster*.

A novel type of respirometer was developed by Heatley, Berenblum, and Chain (*37*), which permits the measurement of gas exchanges of the order of 1 cu. mm. per hour with a systematic error not greater than that involved in the usual manometric methods. The material to be studied is enclosed in a glass chamber equipped with a mica diaphragm. The pressure changes taking place in the vessel are compensated by external pressure applied by a manometric device. The point of compensation is determined with the aid of a sensitive optical indicator system involving a pair of small mirrors attached to the mica diaphragm. Gerard (*31*) modified the usual form of Warburg apparatus by immersing in the thermostat the entire manometer including all gas space, thus permitting the use of smaller vessels with a consequent increase in sensitivity.

The new Fleischmann-Aminco fermentometer (*70*), shown in Figure 7, appears to be the most recent development and modification of the Warburg apparatus. This instrument has been used by a number of laboratories, principally for the determination of vitamin B_1 (thiamine) according to the yeast fermentation method (*71*, *72*). It may be used, however, in any experimental work in which it is desired to collect the gas from a fermenting solution (*73–75*). The rate of fermentation is measured by the amount of gas evolved in a given time interval. The fermentometer presents no new departures in principle from other forms of manometric apparatus, nor does it achieve new values in accuracy, sensitivity, or ability to deal with minute quantities of material.

Linderstrøm-Lang (*53*) first suggested the use of the Cartesian diver, illustrated in Figure 8, as an ultramicromanometer and details of the method were presented in a later communication (*54*). A thorough discussion of the principle, technique, and procedure for making the divers will be found in the work of Boell, Needham, and Rogers (*11*). The delicacy of the instrument and method can be readily appreciated by the fact that whereas 1 cm. on the Warburg manometer scale corresponds to a gas exchange of about 20 cu. mm., 1 cm. on the diver manometer scale corresponds to a gas change of about 0.008 cu. mm. The actual sensitivity of the Cartesian diver is approximately one-fifth of this value; it is 1500 times more sensitive than the standard Warburg manometer. This instrument has been successfully used for determining the carbon dioxide output, ammonia output, and the respiratory quotient in morphogenesis and metabolism studies of amphibian gastrula.

Another modification of the Warburg manometer, of particular interest to the organic chemist, is the apparatus developed by Kuhn and Moller (*50*) for the exact determination of the number of double bonds of highly unsaturated or very slowly hydrogenated substances. The method described measures the hydrogen used up by the substance against a comparison substance under exactly similar conditions. The accuracy attained is such that after hydrogenation for a period of 40 hours an error of ±0.5 per cent is not exceeded.

TABLE I. SENSITIVITY OF MICRORESPIROMETERS

Apparatus	Gas Change per Cm. Manometer Scale, Cu. Mm.	Author	Reference	Date
Standard Warburg manometer	20	Warburg	(*95*)	1926
		Dixon	(*20*)	1934
Warburg micromanometer		Laser	(*52*)	1932
	3–7	Meier	(*57*)	1931
Fenn volumeter	3–4	Fenn	(*28*)	1927
			(*29*)	1935
		Schmitt	(*69*)	1933
		Duryee	(*24*)	1936
Capillary micromanometer	1.8–2.3	Gerard and Hartline	(*32*)	1934
		Victor	(*90*)	1935
	0.06–0.12	Stefanelli	(*82*)	1937
	0.001 (sensitivity)	Tobias and Gerard	(*88*)	1941
Krogh-micromanometer	1.6	Bodine and Orr	(*10*)	1925
Differential volumeter	0.6	Thimann and Commoner	(*86*)	1940
Micromanometer with mica mirrors optically read	0.08–0.04	Heatley, Berenblum, and Chain	(*37*)	1939
Cartesian diver ultramicromanometer		Linderstrøm-Lang	(*53*)	1937
	0.008–0.02	Linderstrøm-Lang	(*54*)	1938
	0.001 (sensitivity)	Boell, Needham, and Rogers	(*11*)	1939

A comparison of the sensitivity of the various microrespirometers mentioned is presented in Table I. The amount of gas exchange per centimeter on the manometer scale is given for each type of apparatus.

Accuracy and Precision of Results

In discussing the accuracy of the Warburg techniques, it may be said that the readings are usually significant to the nearest 0.5 mm., thereby permitting the detection of volume changes of the order of 0.5 μl. (0.0005 ml.). However, by means of certain refinements and modifications, such as the use of special micromanometers with a fine capillary bore (less than 1 sq. mm.) and vessels of smaller capacity (less than 5-ml. volume), and by making microscopic manometric readings, this delicacy of measurement may be increased some fifty to one hundred times.

It has been estimated by Dickens and Simer (*19*) and Dixon (*20*) that the total errors inherent in manometric methods of this type usually amount to approximately 2 per cent. The determination of the vessel constant is probably the most accurate part of the technique. With the standard style of Barcroft differential manometer it is possible to determine the apparatus constant, k, according to Equation 2 with an accuracy of at least 0.5 per cent. When a vessel constant becomes as large as 10, the accuracy with which the reading may be interpreted becomes only 0.1 as great as when the constant is 1, and each graduation will represent an internal change of 10 and 1 cu. mm., respectively.

Singh and Mathur (*76*), using a Barcroft respirometer, found the accuracy of the apparatus to be ± 1.25 per cent, when used for quantitative determinations of known amounts of carbon dioxide liberated by adding measured amounts of standard acid from the vessel side arms to an excess of a bicarbonate solution contained in the main part of the vessel. When applied to the determination of a specific substance, such as carbon monoxide in blood, the Barcroft-Warburg apparatus compares favorably with the well-known referee method of Van Slyke and Neill (*89*). The average discrepancy between these two methods according to Roughton (*66*) was found to be 0.06 volume per cent carbon monoxide (= 0.3 per cent COHb), and in over thirty replicates by the Barcroft method the average discrepancy was 0.12 volume per cent carbon monoxide (= 0.6 per cent COHb).

Johnston and Frey (*43*) found that by using the same reaction vessel for each measurement and carefully controlling conditions, a precision of 1 per cent could be easily attained with the Barcroft-Warburg equipment in measuring the autoxidation induction periods of fatty oils. This study presents an interesting feature in that the determinations were made at 100° C., thus indicating that these methods can be extended to elevated temperatures with convenience and relatively good precision. Corbet and Wooldridge (*18*) investigated the accuracy of the Barcroft differential manometer in respiration studies and concluded that single or even duplicate experiments should be accepted with caution. The experiments should be replicated sufficiently and continued for long enough periods to give a standard error of 5 per cent. The direct method of Warburg in metabolism experiments has been criticized by Brock, Druckrey, and Richter (*13*) on the grounds that when intensive respiration is to be expected (as in the oxygen consumption of eggs) the manometer drops slightly or rises but very little, thus indicating the formation of some compensating gas. The source of error was found to be carbon dioxide formed from sodium bicarbonate by fixed acids, the absorption of carbon dioxide not being instantaneous as is generally assumed.

In summarizing the ultimate factors affecting the accuracy, sensitivity, and speed of response of the Barcroft-Warburg apparatus may be mentioned the limitations arising from:

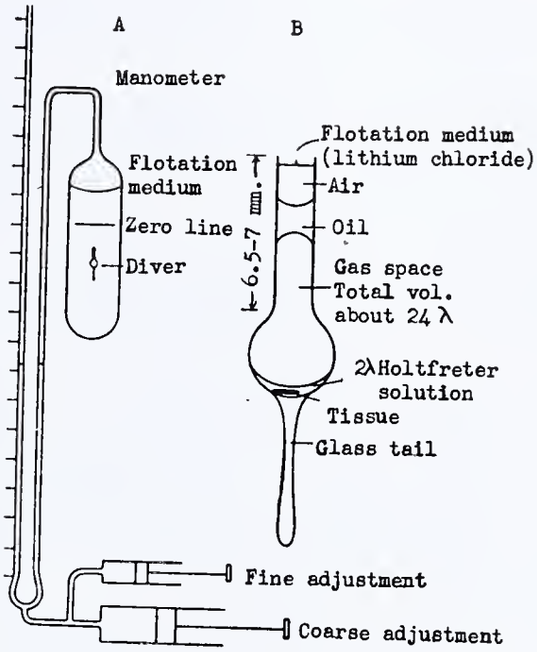

FIGURE 8. CARTESIAN DIVER (B)

Inability of the oxygen to diffuse with sufficient rapidity from the gas into the liquid phase.

Speed of shaking—rate of uptake must be independent of rate of shaking.

Insufficient rate of absorption of carbon dioxide by the alkali present (see work of Dixon and Elliott, *22*).

Use of flasks of improper design and shape, thereby inhibiting free diffusion of gas into liquid.

Diffusion of gases through ground-glass joints.

Practically all the methods employing the Barcroft-Warburg apparatus are based upon the assumption that oxygen and carbon dioxide are the only gases produced or absorbed. Such a statement may be accepted as correct except in the case of certain bacteria, which may evolve hydrogen and other gases; in these instances the methods may be of little value. The presence of excretory nitrogen has also been shown to exert a marked effect on the measurements of the respiratory quotient of single cells or small groups of cells (*80*).

Methods of Measuring Respiration

The present manometric methods for measuring oxygen consumption may be classified into two groups—namely, those in which the oxygen uptake is measured directly, and those in which it is obtained by indirect calculation. (A detailed account of the methods developed by Warburg will be found in the excellent review by Krebs, *48*.) The direct method (*14, 17*) is, perhaps, the most widely used because of its simplicity and suitability for following the course of the oxygen absorption of a substance.

It is assumed that oxygen is consumed and carbon dioxide is produced during the course of the experiment. The presence of alkali in the inset (central well) of the respiration vessel (see Figure 1) keeps the carbon dioxide tension at zero. A decrease in pressure in the vessel is converted into an equivalent volume assumed to be that of the oxygen consumed. The comparison of pressure values obtained in the manner indicated above and those obtained similarly from a vessel in which no alkali is present gives, presumably, the increase in pressure due to the liberation of carbon dioxide, and these values are in turn converted into the equivalent volumes of carbon dioxide produced.

The indirect method (*1, 92, 94, 96*), on the other hand, indicates the total amount of respiration during a definite period, and enables the oxygen to be measured in bicarbonate solutions,

and by working out the results for a number of successive periods, the course of the reaction can be followed.

The respiratory carbon dioxide is not absorbed in this technique but advantage is taken of the fact that carbon dioxide is more readily soluble than oxygen in water. Therefore, the manometer readings will be the resultant of the oxygen absorption and the carbon dioxide production. Three Barcroft or Warburg manometers and vessels are required in this method for a single experiment, one set being used as a thermobarometer. The other two vessels contain equal amounts of the respiring material suspended in unequal amounts, 3 and 8 ml., respectively, of a fluid medium such as 0.025 *M* bicarbonate-Ringer solution or the physiological salt solution recommended by Krebs and Henseleit (*49*).

If the sample of material consists of excised tissue slices, these may be sectioned to the proper thickness (4.7×10^{-2} cm.) according to Warburg's technique (*93*), or by the method of Thomas and DeEds (*85*), usually, 20 to 50 mg. of moist weight are taken. Samples such as a homogeneous suspension of yeast cells or bacteria may be prepared in bicarbonate-Ringer solution and the two different volumes measured directly into the vessels. The flasks used in this particular technique must be equipped with venting plugs and outlets for passing in a gas mixture consisting of 95 per cent oxygen and 5 per cent carbon dioxide. This not only keeps the bicarbonate-Ringer solution in equilibrium with the gas mixture but prevents the precipitation of calcium carbonate as the solution becomes alkaline upon exposure to the air.

If h and H are the respective readings for the same time interval, and assuming that the respiration rate of the sample is the same in both manometers, the following relationship may be expressed, where k and K are the respective vessel constants:

$$\text{Oxygen uptake} = \frac{hk_{CO_2} - HK_{CO_2}}{\dfrac{k_{CO_2}}{k_{O_2}} - \dfrac{K_{CO_2}}{K_{O_2}}}$$

$$CO_2 \text{ evolution} = \frac{hk_{O_2} - HK_{O_2}}{\dfrac{k_{O_2}}{k_{CO_2}} - \dfrac{K_{O_2}}{K_{CO_2}}}$$

By the application of the same principle, carbon dioxide assimilation as compared with respiration, fermentation as compared with respiration, and butyric acid fermentation as compared with lactic acid fermentation can be measured manometrically. The indirect method has been of considerable value in the study of animal tissue respiration under physiological conditions, especially in the investigation of tumors. It has been improved upon by Dickens and Simer (*19*) and more directly by Dixon and Keilin (*23*).

A brief outline of the various steps involved in making concurrent measurements of the oxygen consumption and carbon dioxide production according to the direct method may be given as follows:

1. Determine experimentally the time periods required for temperature adjustment. (This can be done by placing the vessels in the bath under experimental conditions and observing the time necessary to bring the manometric fluid to a constant level with the stopcock of the manometer closed.)
2. Place in the side arm of the flask a definite amount of a dilute acid, usually 0.5 ml. of *N* hydrochloric acid, and 0.2 ml. of 30 per cent potassium hydroxide in the adjacent side arm or the central well (inset) of the flask. (This step of the procedure can be modified to meet the individual requirements of the material being studied and will vary with the type of vessel used.)
3. Assemble the manometer and flask and attach to the shaking mechanism, thus submerging the flask in the thermostat. Shake for about 10 minutes to allow the system to become equilibrated at the desired temperature.
4. Remove the unit from the shaker, detach flask, and insert sample of respiring material into main compartment of vessel. Then reassemble the unit and return it to the shaker. Record time *A* as soon as the sample is placed in the vessel and regard this as the beginning of the period of carbon dioxide production.
5. Shake for 3 minutes to allow respirometer to reach temperature of bath, then adjust indicating fluid to the 150-mm. mark, and note reading *B*, on the open arm of the manometer, together with time *C*. Close stopcock. *C* is regarded as the beginning of the period of oxygen consumption. The apparatus is then allowed to operate for the duration of the experiment.
6. Record time *D* at the termination of the oxygen consumption period and then adjust the manometer fluid to the 150-mm. mark, followed by reading *E*. The length of the oxygen consumption period will then be $D - C$; the amount of oxygen consumed will be represented by $B - E$.
7. Remove manometer from shaker and turn the unit in such a position as to permit the acid in the side arm of the vessel to flow into the alkali, thus liberating the carbon dioxide absorbed. Return manometer to thermostat and shake again for 3 minutes. Adjust indicating fluid, followed by reading *F* and time *G*. *G* marks the end of the period of carbon dioxide production.

In making a series of determinations with the Warburg respirometer, a control unit without respiring material (thermobarometer) must be subjected to the same conditions, reagents, and treatment as the series. This provides a correction for any temperature and external pressure changes during the experiment and for any dissolved carbon dioxide in the reagents.

Consideration must also be given to certain other factors if the above-mentioned methods are to represent the true respiration of the tissue slice or cell suspension in question. As previously stated, the kinetics of gas-liquid reactions can

Table II. Applications

Material Studied	Function Measured	Apparatus Used	Accuracy	Reference	Date
Amino acids	CO_2 evolved	Warburg	...	(*68*)	1938
Arginase (blood)	CO_2 evolved	Warburg	0.003 mg. urea	(*97*)	1934
Ascorbic acid oxidation	O_2 uptake	Barcroft	...	(*7*)	1936
Bacterial growth	O_2 uptake	Warburg	...	(*36*)	1941
Beer oxidation	O_2 consumed	Warburg	...	(*51*)	1940
Carboxylase	CO_2 evolved	Dixon-Keilin	...	(*35*)	1941
Catecholase	O_2 absorption	Barcroft	±2–3%	(*58*)	1941
Coenzyme I (tissues)	CO_2 evolved	Warburg	10%	(*3*)	1939
Coffee oxidation	O_2 absorbed	Warburg	...	(*42*)	1938
Cozymase	CO_2 evolved	Warburg	±0.03 μg.	(*40*)	1941
Enzyme purification	O_2 uptake	Warburg	...	(*4, 9, 27*)	1935
Fatty acid oxidation	O_2 uptake	Warburg	...	(*8, 59*)	1938
Fatty oils oxidation	O_2 absorption	Warburg	1%	(*43*)	1941
Germicides (evaluation)	O_2 uptake	Warburg	...	(*36*)	1941
Glutathione (tissues)	CO_2 production	Warburg	<6%	(*100*)	1935
Glyoxalase	CO_2 production	Warburg	2%	(*60*)	1934
Gases (microanalysis)	N_2 fixation				
	O_2 absorption	Warburg	...	(*15*)	1932
Grains (cereal)	CO_2/O_2	Warburg	...	(*84*)	1942
Hydrogen transport (mechanism)	O_2 uptake	Warburg	...	(*61*)	1940
Hydrogenations (microcatalytic)	H_2 absorption	Barcroft (mod.)	±2%	(*39*)	1930
Insulin and carbohydrates	R. Q.	Warburg	...	(*38, 81*)	1940
Narcotics (brain tissue)	QO_2 absorption	Barcroft	...	(*63*)	1934
Nitrifying bacteria	O_2 consumption	Warburg	...	(*12*)	1939
Phenolase (plant tissues)	O_2 absorption	Warburg	2.5%	(*67*)	1935
Photosynthesis (algae)	O_2/CO_2	Warburg	...	(*30*)	1940
Sewage effluents	O_2 absorption	Barcroft	...	(*101*)	1936
Sulfonamide compounds (bacterial metabolism)	O_2 consumption	Warburg	...	(*47*)	1940
Soils (CO_2 content)	CO_2 production	Barcroft	...	(*79*)	1932
Tea fermentation	O_2 uptake	Warburg	...	(*64*)	1941
Thiamine (biological materials)	CO_2 production	Warburg	102–105% recovery	(*33*)	1941
		Fermentometer	2%	(*70*)	1942
		Warburg-Summerson	2%	(*44*)	1942
Tubercle bacilli (metabolism)	O_2 consumption	Warburg	...	(*46*)	1939
Unsaturated compounds	H_2 absorption	Warburg	0.5%	(*50*)	1934
Urease activity	CO_2 production	Warburg	...	(*78*)	1941
Volatile substances soluble in water	Ether	Warburg	...	(*45*)	1937
Wheat respiration	O_2 uptake	Fenn	...	(*2*)	1938
Wood sections	O_2 consumption	Fenn	...	(*34*)	1940

be recorded manometrically only if the rates so observed are independent of the speed of shaking of the vessel and of the relative volumes of the liquid and gas phases. Otherwise, the observed rates are dependent upon the speed of diffusion of dissolved gas between the two phases and also on the true speeds of the chemical processes.

Allowance can be made for the effect of diffusion in manometric experiments by assuming the existence of a stationary film of liquid, at the boundary between the liquid and gas phases, diffusion through which determines the rate of exchange of gas between gas and liquid. Roughton (*65*) has developed an excellent method of allowing for the influence of diffusion in manometric measurements by carefully studying the kinetics of carbon dioxide uptake and output by buffer solutions, in the presence of carbonic anhydrase. The method is based upon the stationary film theory of gas-liquid interchange which states that when solution of a gas occurs without any chemical reaction

$$\text{Rate of gas uptake} = \frac{D_i}{\delta} A\,(C_i - C_L) \qquad (3)$$

where

D_i = diffusion coefficient of dissolved gas
δ = thickness of stationary film
A = area of stationary film in sq. cm.
C_i = concentration of dissolved gas at the outer surface of the stationary film $= \alpha P_i$
α = solubility coefficient of the gas in the liquid
P_i = pressure of the gas in atmospheres
C_L = average molal concentration of the dissolved gas in the bulk of the liquid phase

Equation 3 is also valid for the liberation of dissolved gas from liquid.

Applications

The applications and general usefulness of the Barcroft-Warburg apparatus as an analytical tool for biological and chemical research can hardly be overemphasized. While the greater majority of workers utilizing this instrument have been interested chiefly in purely biochemical or physiological problems, it nevertheless has been and can be used to considerable advantage by workers in allied fields. Typical examples of the diversified applicability of this equipment will be found in Table II. It is not the purpose of this paper to present a complete bibliography, but rather to provide a list of the more important key references and thereby assist the analyst and potential user to select those methods particularly suited to his problems.

Acknowledgment

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Microdetermination of Carbon in Steels

Modification of the Standard Carbon-Hydrogen Train

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IN JUNE, 1940, the Metallurgical Section of this laboratory asked the authors to determine low percentages of carbon in small samples of Nichrome for the purpose of establishing whether certain segregations formed in this alloy during service were carbides or nitrides.

Since the amounts of carbon involved were far too small for the macrocombustion method, the authors developed a micromethod by introducing a quartz furnace into the standard carbon-hydrogen train (*2*) just before the Supremax combustion tube (universal filling). A modification of some sort is necessary because combustion in ordinary microanalysis occurs at temperatures far below the minimum of 1100° to 1150° C. advisable in the case of steels. More recently, Smoluchowski (*3*) requested carbon determinations on small samples to corroborate routine analytical results obtained in connection with experiments on the diffusion of carbon in steel. For this work, the authors modified the standard microtrain in another way—by slipping a furnace directly over the first part of a standard quartz combustion tube.

Both modifications eventually gave satisfactory results and promise to be particularly useful when such carbon determinations are made only rarely, so that the setting up of a special train is not warranted. The micromethod of Klinger, Koch, and Blaschczyk (*1*) requires a special train.

Experimental

Auxiliary Combustion Tube. The quartz auxiliary combustion tube (Figure 1) was calibrated with a thermocouple in the position of the boat, *G*. Standard microchemical technique was observed throughout except for the following details. Granulated tin served as accelerator. A weight of tin equal to half the weight of sample sufficed, although much more was occasionally added. Before steel samples were run, the train was conditioned by burning an unweighed primer of sugar in the conventional way. The auxiliary combustion tube was heated to 900° C. in 10 minutes and to 1150° C. in 25 minutes. The steel sample usually began to burn with a bright glow 3 or 4 minutes after heating was begun. After 30 minutes of heating time had elapsed, the current was interrupted, and the train was flushed for 20 minutes more. An oxygen flow of 5 cc. per minute was maintained for the entire 50-minute period; during the combustion of the larger samples, the rate at which oxygen was introduced had to be increased in order to maintain this constant flow. Suitable blank corrections were determined from time to time on boats containing tin. These corrections, usually near 40 micrograms of carbon dioxide, were applied to the analytical results, which are given in Table I.

The reliability of the method is shown by the last three results, which were obtained on a standard low-carbon steel. Furthermore, the carbon content of the Nichrome samples

Table I. Results with Auxiliary Combustion Tube
(Small Nichrome samples)

Sample	Sample Weight	Tin	Carbon
	Mg.	*Mg.*	%
318A	4.19	34	0.4
322D	3.84	32	1.3
325G	4.10	37	1.1
326A	4.00	24	2.0
	2.24	44	2.0
	2.14	20	2.1
	3.81	36	2.2
327D	3.95	33	0.7
	3.75	36	0.7
328C	4.40	29	1.8
	2.99	32	1.6
329F	4.49	37	2.5
51690[a]	23.8	24	0.24
	46.0	46	0.26
	20.6	19	0.26

[a] 0.28% C by routine macromethod on this low-carbon steel.

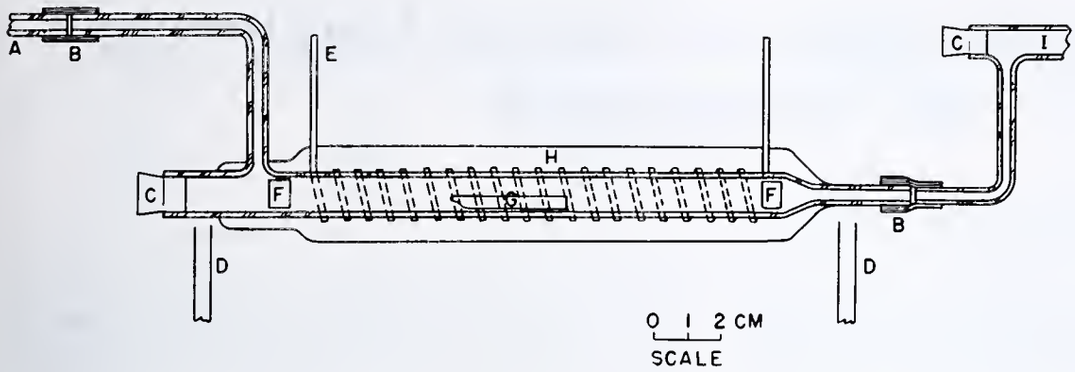

FIGURE 1. CROSS SECTION OF AUXILIARY (QUARTZ) COMBUSTION TUBE

A. Bubble counter
B. Impregnated tubing
C. Corks faced with Al foil
D. Air jets
E. Nichrome tape, 0.025 × 0.16 × 84 cm.
F. Gold foil baffles
G. Porcelain boat
H. Asbestos tape
I. Microcombustion tube

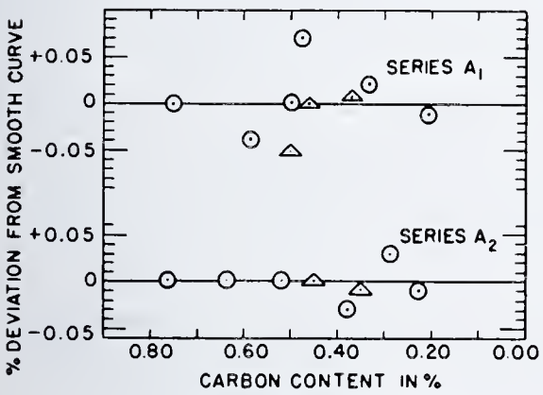

FIGURE 2. PLOT OF GRAPHICALLY OBTAINED DEVIATIONS

△ Micro results
⊙ Macro results

paralleled the amount of the segregated phase shown by the corresponding photomicrographs. This phase could have been only a carbide or a nitride, and nitrogen analyses proved this element to be virtually absent.

When the work was begun, devitrification of quartz and failure of the Nichrome heating units were expected; 20 hours of operation between 900° and 1150° C., however, did not impair the usefulness of the furnace.

SLEEVE FURNACE. In the hope of simplifying the apparatus, a platinum-wound furnace (9 cm. long) was constructed so that it could be slipped over the end of a standard quartz microcombustion tube with 0.5-mm. clearance. During the combustion period, this furnace was placed 0.5 cm. from the entrance end of the long furnace in the microtrain; the sample boat was thus in the position prescribed for the regular carbon and hydrogen microanalysis. A platinum baffle and an air jet were used to protect the cork at the entrance of the combustion tube. With this sleeve furnace, 1150° C. was reached in 5 minutes and maintained for 25 minutes more; otherwise the manipulation was identical with that described above.

The authors soon discovered that the standard quartz combustion tube (walls about 0.8 mm. thick) was too thin for operation at 1150° C. After one failure due to external devitrification, quartz of 1.5-mm. wall thickness was used for the first section of the tube; operation thereafter was satisfactory. The results obtained with the sleeve furnace are given in Table II.

In the analysis of the diffusion specimens, high accuracy was sought. Since the specimens for the microdetermination were taken from between others on which careful macroanalyses had been done, it proved possible to compare the two methods by measuring the deviations from a smooth curve drawn through the carbon contents of all the samples (Figure 2).

The accuracy of the results in Table II can be summarized on the basis of the average difference between the amount of carbon found and the carbon content of the sample calculated from the per cent obtained on the smooth curve referred to above. This average difference for the first ten results in Table II amounts to only 13 micrograms of carbon, in spite of the fact that absolutely uniform samples could not be obtained. If the worst value (0.488 per cent on Sample A1F) is discarded, the average difference for these ten results drops to 7 micrograms of carbon. The average difference between the carbon found and the true value for the Bureau of Standards sample is 3 micrograms of carbon. On the basis of the latter figure, 0.1 per cent carbon in a 100-mg. sample of steel can be determined within ±0.003 per cent.

TABLE II. RESULTS FOR DIFFUSION SPECIMENS OF ORDINARY LOW-CARBON STEEL

Sample	Sample Weight	Tin	Carbon	Average Carbon
	Mg.	*Mg.*	%	%
A1F	17.15	30	0.514	0.501
	106.5	50	0.488	
A1G	25.97	30	0.471	0.465
	100.1	50	0.459	
A1H	103.9	50	0.345	0.354
	105.0	50	0.363	
A2D	105.6	50	0.445	0.449
	102.2	50	0.453	
A2E	104.9	50	0.344	0.347
	49.90	25	0.349	
B. S. 13C[a]	14.12	30	0.539	
	102.9	50	0.574	
	105.3	50	0.571	

[a] Bureau of Standards sample = 0.573% C.

Summary

1. The standard carbon-hydrogen microapparatus can be readily adapted to the microanalysis for carbon in low-carbon steels or Nichromes.
2. In a 100-mg. sample of steel 0.1 per cent carbon can be determined within ±0.003 per cent.
3. The method is particularly useful where only a few samples are to be analyzed at rare intervals.
4. No attempt was made to speed up the determinations.
5. The usual quartz combustion tubes are not suitable for continued use at 1150° C.

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Ultramicromethod for Sodium Employing the Polarograph

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IN INVESTIGATIONS of the chemical composition of isolated mouse epidermis, and of that epidermis undergoing carcinogenesis by methylcholanthrene (*3*, *7*), the very small amounts of tissue available necessitated the adoption of micromethods for the determination of most constituents. This was especially true for sodium. For that metal, a polarographic technique was devised which depends upon the fact that sodium zinc uranyl acetate in dilute hydrochloric acid solution can give well-defined diffusion currents which are directly proportional to the concentration of the triple acetate salt.

Apparatus and Reagents Used

Heyrovský polarograph, model XI (E. H. Sargent & Co.).

Redistilled water for sodium standards, washing, and other operations, prepared in an all-glass (Pyrex) apparatus.

Zinc–uranyl acetate solution, prepared according to the method of Barber and Kolthoff (*1*). This solution was saturated with the triple acetate salt and filtered just before use through a sintered-glass disk covered with a layer of asbestos.

Wash solution, made by saturating 95 per cent alcohol with sodium zinc uranyl acetate, and filtering immediately before using as described above.

Asbestos ("Italian washed and ignited"), ground fine in a mortar, washed on a sintered-glass filter, and suspended in redistilled water.

Experimental

The calibration data (Table I) were obtained from solutions of carefully prepared sodium zinc uranyl acetate in 0.5 *N* hydrochloric acid. The values m and t (where m is the rate of flow of mercury from the capillary, expressed in mg. per second, and t is the drop time in seconds, *4*) were measured in 0.5 *N* hydrochloric acid at a potential of −0.6 volt with respect to the saturated calomel electrode. The diffusion current, i_d, was found proportional to the concentration, C, of sodium zinc uranyl acetate over a range of 0.1625 to 0.975 millimolar, with or without caffeine. This compound easily suppressed occasional maxima. These were, however, of such rare occurrence that the diffusion currents could be measured with or without the alkaloid. The diffusion current and the ratio i_d/C for any particular concentration were significantly less in the presence of caffeine. At the dropping mercury electrode, uranium is probably reduced from a valence of 6 to 4. It was impossible to measure accurately the half-wave potential with the author's instrument, but it was between −0.22 and −0.26 volt with respect to the saturated calomel electrode. Strubl (*6*) found that solutions of uranyl salts in 2 *N* hydroxylamine hydrochloride gave a well-defined wave with a half wave potential of −0.24 volt. The diffusion current was directly proportional to the concentration of the uranyl salts.

Procedure for Solutions

Solutions free of phosphate and organic material, and containing only small amounts of potassium, were analyzed as follows:

After evaporation to dryness on a low-temperature hot plate in 50-cc. Pyrex beakers (cleaned with chromic acid and rinsed with redistilled water), the residues were taken up in 4 to 6 cc. of zinc–uranyl acetate reagent and allowed to stand at nearly constant temperature for several hours (or overnight) to complete the precipitation. This was carried out beneath a large inverted crystallizing dish, under which was also placed a beaker of water to minimize evaporation. When precipitation was complete, the supernatant fluid was removed by the filter-stick method of Lindner and Kirk (*5*). For this purpose, Pyrex immersion filter tubes (10-mm. disk) of medium porosity were used. The precipitate was washed with several 2-cc. portions of wash alcohol solution.

The sodium zinc uranyl acetate in the beaker and on the filter stick asbestos pad was left at room temperature until dry. Then, an accurately measured volume of 0.5 *N* hydrochloric acid was added to dissolve the triple acetate salt. The amount of acid used had to be sufficient to keep the diffusion current in the range of the calibration curve. It was not difficult to estimate this amount of hydrochloric acid after some trials. The solution was passed through filter paper (to remove the asbestos) into small "shell vials", and a trace of caffeine (Merck's anhydrous powder) was added. Shell vials proved to be excellent containers for the electrolysis, since the various sizes available readily accommodated different amounts of solution, and the dropping mercury electrode could be placed well below the surface of the liquid to maintain a good degree of submersion. After removing the oxygen by a stream of nitrogen bubbles, the diffusion current was measured. From this the sodium content could be read directly from the calibration curve.

Typical recoveries from pure solutions of sodium chloride are shown in Table II. The accuracy for the amounts of sodium taken was ±0.7 to ±1.5 per cent. Since a 0.1625 millimolar solution of sodium zinc uranyl acetate (equivalent to 3.47 micrograms of sodium per cc.) has a diffusion current greater than one microampere, it is obvious that the method can be used for determining mere traces of sodium.

Procedure for Tissues

In biological materials, interfering substances other than phosphate and organic compounds are rarely found in effective concentrations (*5*). The following procedure has been

TABLE I. CALIBRATION DATA FOR SODIUM IN SODIUM ZINC URANYL ACETATE

Various concentrations of sodium zinc uranyl acetate in 0.5 *N* hydrochloric acid at 25° C. Air removed from solutions with nitrogen. h = 65 cm. Diffusion currents measured at a potential of −0.6 volt with respect to saturated calomel electrode. With caffeine t = 3.385 sec., $m^{2/3}t^{1/6}$ = 2.794 mg.$^{2/3}$ sec.$^{-1/2}$. Without caffeine t = 3.512 sec., $m^{2/3}t^{1/6}$ = 2.904 mg.$^{2/3}$ sec.$^{-1/2}$.

Sodium Zinc Uranyl Acetate		i_d				i_d/C	
		Observed		Corrected			
Millimolar	Sodium equivalent	With caffeine	Without caffeine	With caffeine	Without caffeine	With caffeine	Without caffeine
	Micrograms/cc.	*Microamperes*		*Microamperes*		*Microamp./mmole/l.*	
0.0	...	0.200[a]	0.209[a]	....	....	..	..
0.1625	3.47	1.239	1.348	1.039	1.139	6.39	7.01
0.260	5.98	1.871	.....	1.671	....	6.42	..
0.325	7.47	2.324	2.629	2.124	2.420	6.55	7.44
0.487	11.21	3.384	3.707	3.184	3.497	6.54	7.18
0.650	14.95	4.556	5.026	4.356	4.817	6.69	7.41
0.975	22.43	6.827	7.336	6.627	7.127	6.79	7.31
1.30	29.90	9.302	10.337	9.102	10.128	7.00	7.79
2.60	59.80	19.094	21.270	18.894	21.062	7.26	8.10
3.25	74.80	24.289	27.378	24.089	27.169	7.41	8.36

[a] Residual current of supporting electrolyte alone.

Table II. Assay of Known Sodium Chloride Solutions

Sodium Added	Recovered[a]	Difference
Mg.	*Mg.*	*Mg.*
0.0590	0.0598	−0.0008
0.1574	0.1560	−0.0014
0.1970	0.2000	+0.0030
0.2360	0.2380	+0.0020
0.3147	0.3124	−0.0023
0.3934	0.3960	+0.0026

[a] Values after correcting for blanks.

used for the determinations of sodium in 100 to 400 mg. (wet weight) of epidermal tissue.

The samples were ashed at 450° C. (a temperature which has been shown, *5*, to result in no loss of sodium) in a muffle furnace until all the organic matter was destroyed. The ash was then dissolved in 1 cc. of 0.1 *N* hydrochloric acid and this solution washed out of the crucibles into 15-cc. centrifuge tubes with several 2-cc. portions of redistilled water. Phosphate was removed by the method of Butler and Tuthill (*2*) as follows: Calcium hydroxide powder was added to the solutions in the centrifuge tubes, after which they were stoppered and allowed to stand with occasional shaking for 2 hours. The tubes were centrifuged and the supernatant liquid was decanted into clean 50-cc. Pyrex beakers, 2-cc. portions of water were added to each centrifuge tube, the stoppers returned, and the tubes agitated until the calcium hydroxide was resuspended. Centrifugation was repeated, and the supernatant wash fluid was decanted into the respective beakers. Three washings of the calcium hydroxide were carried out. The phosphate-free solutions were evaporated to dryness and the sodium content was determined as described above. Blanks on the hydrochloric acid, water, and calcium hydroxide were necessary, especially for the last-mentioned reagent.

Summary

It has been shown that 0.1625 to 0.975 millimolar solutions of sodium zinc uranylacetate in 0.5 *N* hydrochloric acid give well-defined polarographic diffusion currents. These currents are directly proportional to the concentration of the triple acetate salt. The use of this technique permits the determination of exceedingly small amounts of sodium as in limited samples of biological material.

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Electrographic Detection of Molybdenum in Steel Alloys

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MOLYBDENUM may be detected rapidly by spot tests (*4*, *5*) and tests have been described for detecting molybdenum in steel alloys (*1*). A very rapid electrographic test for molybdenum and molybdenum in steel alloys is described here, which may be completed in a few seconds.

When molybdenum or molybdenum alloy is made the anode in a neutral or slightly acid solution of nitrate ion, using an e. m. f. of 6 to 9 volts and a current of 0.5 to 1.0 ampere, the anode becomes passive (*2*) and the molybdenum and iron enter solution as molybdate and ferric ion, respectively. When a hydrochloric acid solution of stannous chloride and potassium thiocyanate is added, molybdenum forms a complex trivalent molybdenum thiocyanate, yielding a carmine red solution (*4*). Trivalent iron is reduced to divalent by divalent tin. When the test is carried out electrographically, the molybdate is precipitated as lead molybdate with lead acetate solution, which also reduces the concentration of other soluble heavy metal ions, thereby increasing the sensitivity of the test.

Apparatus and Test Solutions

The equipment and procedure are identical with those described in electrographic tests (*2*, *3*).

Test Solution A. Dissolve 30 grams of sodium nitrate, reagent grade, in sufficient distilled water to make 100 ml. and filter.

Test Solution B. Dissolve 1 gram of lead acetate, A. C. S., in sufficient distilled water to make 100 ml. and filter.

Test solution C. Dissolve 30 grams of stannous chloride, A. C. S., in sufficient hydrochloric acid, A. C. S., to make 100 ml. and add to an equal volume of molar potassium thiocyanate. It is advisable to mix only sufficient quantity of the two solutions to last 24 hours.

Method

Fold ashless filter paper to form a wad three to four layers thick and then dip into test solution A. Place the moistened filter paper on the test metal anode and press a graphite cathode about 7.5 cm. (3 inches) long and 0.6 cm. (0.25 inch) in diameter firmly against the free side of the moistened section of the filter paper for about 1 second. An e. m. f. of about 6 to 9 volts is employed and may be obtained by using 4 to 6 No. 6 dry cell batteries connected in series. The current is about 0.5 to 1.0 ampere. A contact of about 3 seconds is recommended for molybdenum steel alloys.

Wash the moistened end of the filter paper with test solution B to remove some of the soluble heavy metal ions and to precipitate the molybdate and fix it to the paper as lead molybdate. Add a few drops of test solution C to the spot on the side of the filter paper that was adjacent to the test metal. The trivalent molybdenum thiocyanate complex formed will yield a carmine red stain.

Molybdenum steel alloys containing 0.2 per cent molybdenum and upwards have been tested. The intensity of the color produced increases with the molybdenum content when the current density and time remain constant. When ferric ion is present, red ferric thiocyanate forms, but ferric iron is reduced quickly to ferrous, which produces a pale green stain that does not interfere with the carmine molybdenum thiocyanate color.

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An Iodoform Microtest for the Higher Alcohols and Ketones

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IN THE study of the lipides of the tubercle bacillus, higher alcohols such as *d*-eicosanol-2 (*1*, *7*) and phthiocerol (*9*) ($C_{36}H_{74}O_3$) have been among the many compounds isolated. To prove the presence or absence of the —CH(OH)CH_3 grouping in these alcohols, it was hoped that the widely used iodoform test might be applied. Unfortunately, the methods heretofore described for carrying out this test were not applicable to these compounds. Scarcity of material or insolubility in water prevented the use of procedures based on the isolation of iodoform for a melting point determination or microscopic study (*2–4*).

These difficulties have now been circumvented by employing the sensitive color test described by Lustgarten (*5*), in which iodoform gives a deep red color when heated with resorcinol and potassium hydroxide. As a solvent for the higher alcohols and ketones, methanol has been found satisfactory. By this method it is possible to detect 1 mg. of eicosanone-2 or 3 mg. of the corresponding alcohol. The fact that the alcohols can be used directly in this test is very helpful in those cases where the amount of material is insufficient to allow oxidation to the ketone. The use of the alcohol is also of advantage in work with compounds such as phthiocerol, which cannot be converted to ketones without disrupting the molecule.

The test was positive with 1-mg. samples of *p*-phenylacetophenone, methyl octadecyl ketone (eicosanone-2), methyl pentadecyl ketone, *o*-benzoxyphenyl acetone, methyl-β-naphthyl ketone, and benzoyl acetone and with 3-mg. samples of *d*-eicosanol-2 and 5,5-dimethyl-2,4-hexanediol. It was negative with citric acid, hydantoin, mannitol, benzophenone, biphenylene oxide, tricarballylic acid, benzyl sulfonamide, and acetyldurene.

Apparatus

The formation of iodoform is carried out in a Pyrex test tube, A, 2.5 cm. in length. This tube is made by fire-polishing the open end (7 mm. in inside diameter) until this dimension is reduced to 4 to 5 mm.

The reaction between the iodoform and resorcinol–potassium hydroxide takes place in a Pyrex test tube, B, 4 cm. long and 7 mm. in inside diameter. A medicine dropper having an opening of 1.5 mm. is used to transfer the contents of tube A to tube B. If the opening is smaller than this, some difficulty may be encountered from solidification of the solution during transfer.

Reagents

Solution I. Three grams of potassium hydroxide are dissolved in 2 cc. of water and 18.7 cc. (15 grams) of pure methanol are added. This solution is stored in a glass-stoppered flask equipped with a medicine dropper.

Solution II. Pure methanol is saturated with iodine with gentle warming. It is kept in the same manner as the alcoholic potassium hydroxide solution.

The Color Test

Blank Test. The reagents are tested as follows: Five drops of solution I are pipetted into tube A, and to this solution solution II is added dropwise until a brown color persists. The tube is held for 5 minutes in a water bath at 60° C. and then cooled to room temperature. Sufficient powdered potassium hydroxide is added to discharge the brown color. This is accomplished by rotating the tube on its side, to make sure that the entire inner surface is moistened.

In tube B are placed a few milligrams of resorcinol, which is then covered with a thin layer of powdered potassium hydroxide. This mixture is made slightly moist by the addition of a small drop of water. Tube B is then heated on an asbestos-covered hot plate until bubbles appear. Without removing the tube from the hot plate, several drops of solution from tube A are at once dropped on the resorcinol–potassium hydroxide mixture. One minute of heating should give only a pale green color.

The material under investigation is tested as follows: Because some compounds discolor on warming with alkali or give a red color with heated potassium hydroxide–resorcinol, it is necessary to carry out a blank on 1 mg. of the compound. The material is placed in a small test tube and warmed at 60° for several minutes with 5 drops of solution I. A slight discoloration does not interfere with the test; a deep color shows the compound to be unsuitable for investigation. Several drops of this solution on addition to heated potassium hydroxide–resorcinol should cause no change in the pale green color of the potassium hydroxide–resorcinol. The compounds used in this study met these requirements.

Test on Ketones. Five drops of solution I are pipetted into tube A, containing 1 mg. of the ketone to be tested. The tube is held in a water bath until the compound is in solution, and solution II is added dropwise until the brown color appears. The tube is placed in a water bath at 60° for 3 to 4 minutes, solution II being added from time to time to restore the brown color. Sufficient powdered potassium hydroxide is finally added to discharge the brown color when the tube is rotated on its side. If considerable iodoform is present, a yellow color may remain.

In tube B are placed a few milligrams of resorcinol, which is covered by a thin layer of powdered potassium hydroxide. A small drop of water is added to make the mixture slightly moist. Tube B is then placed on an asbestos-covered hot plate and when bubbles begin to appear, several drops of the solution in tube A are added. If the compound tested is a methyl ketone, a deep red color is observed in less than 15 seconds.

Test on Alcohols. Eight drops of solution I are pipetted into tube A containing 3 mg. of the alcohol to be tested. The solution is warmed until the compound has dissolved and an excess of solution II is then added. The tube is kept in a water bath at 60° for 3 minutes. By this time, the brown color will have faded, so more of solution II is added to restore it. The solution is heated for another 2 minutes at 60° C. One drop of solution I is added and then an excess of solution II. After another 3 minutes of heating at 60° C., excess powdered potassium hydroxide is added. This solution is added dropwise to moistened potassium hydroxide–resorcinol on the hot plate. If the alcohol is capable of being oxidized to a methyl ketone, a deep red color results in a very short time.

Limitations of the Method

Some halogen compounds [$CCl_3CH(OH)_2$, $CCl_3CHOH.NHCHO$, CCl_3COOH, and $C_6H_2.Cl_3OH$] have been observed by Ware (*11*) to give red colors when heated with resorcinol and potassium hydroxide. In this laboratory $CCl_3.CH(OH)—COOH$ was found to behave similarly; hence care must be taken in the interpretation of results with halogen compounds.

The sterically hindered ketone, acetyldurene, was found to give a negative test. This is to be expected on the basis of the behavior of acetylmesitylene observed by Fuson and Tullock (*4*). For other anomalous haloform reactions, see Suknevich and Chilingaryan (*10*), Slotta and Neisser (*8*), and Paggi (*6*).

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Detection of Zirconium with 5-Chlorobromamine Acid

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BROMAMINE acid is the name used by dye chemists for the sodium salt of 1-amino-4-bromo-2-anthraquinonesulfonic acid. A number of substituted bromamine acids react with the tri- and tetravalent cations in aqueous medium, giving red precipitates. In acid solution, the reactivity is limited to only a few ions, of which the reaction with zirconium is the most sensitive. The chloro derivatives—namely, 5-chloro-, 6-chloro-, and 7-chlorobromamine acid—were found to be the most selective of the substituted bromamine acids studied. Although these three compounds behave similarly, the 5-chloro derivative is recommended because of its slightly more selective reactions. The chlorobromamine acids give heavy bright red precipitates with cadmium, copper, cobalt, nickel, palladium, and zinc in ammoniacal medium.

Although the attempted application of 5-chlorobromamine acid as a quantitative precipitant for zirconium was unsuccessful, the reagent may be used for the detection of zirconium.

Reagents

5-Chlorobromamine Acid. The compound was obtained from E. I. du Pont de Nemours & Co., Inc. A 50 per cent acetone-water solution containing 2 mg. per ml. of the reagent was used.

Zirconium. A stock solution containing 1 mg. of zirconium per ml. and 0.2 *M* with respect to nitric acid was prepared from zirconium nitrate and was diluted with water to the desired concentration.

Nitric Acid. A 2 *M* solution was employed.

Procedure

Transfer 0.05 ml. of the test solution to a depression in a white tile spot plate, and add 0.05 ml. of nitric acid and finally 0.03 ml. of the 5-chlorobromamine acid solution. Shake the spot plate continuously for several minutes before making the observations. A blank prepared in the same manner, except that distilled water is added in place of the test solution, should be used for comparison. Occasionally, the blank may have a small amount of a pale red colored precipitate, which floats on the surface of the liquid and has the appearance of a slight scum. This should not be confused with the precipitate formed in the presence of zirconium, which is more granular and darker colored and settles to the bottom of the solution.

Characteristics of Reaction

The reaction rate is dependent upon agitation of the solution, the concentration of zirconium and of the reagent, and the type and concentration of acid present. Continuous shaking hastens the formation of the precipitate. At the higher concentrations of zirconium the reaction rate is faster than at the lower. The use of a higher concentration of the reagent hastens the precipitation but may cause interference. The reaction rate is about the same in nitric or hydrochloric acid but the latter tends to cause increased interference by the reagent. The reaction rate is greatest at low concentrations of nitric acid and decreases markedly with increasing acid concentration—for instance, using a zirconium concentration of 10 p. p. m. and 0.05 ml. of 0.3 *M* nitric acid, the precipitate forms within a minute; with 2 *M* acid, 2 to 3 minutes are required. The 2 *M* acid is recommended, despite the slower reaction rate, to avoid interference by a number of ions.

Table I. Limiting Concentration

Ion	Limiting Concentration *Mg./ml.*
Na^+	20
Ba^{++}, Ca^{++}, Sr^{++}, K^+	10
Cd^{++}, Co^{++}, Cu^{++}, Mg^{++}, Mn^{++}, NH_4^+	5
Ni^{++}, Pb^{++}, Zn^{++}	5
Rare earths*, Fe^{+++}, Ga^{+++}, Hg^{++}, In^{+++}, Sc^{+++}, Y^{+++}	1
Ce^{++++}	0.03
Be^{++}	0.25
Al^{+++}, Cr^{+++}, Th^{++++}, Ti^{++++}	0.1
PO_4^{---}, SO_4^{--}	1

* Trivalent ions.

Interference

In testing for the interference of the various ions, the procedure previously given was followed using a zirconium solution containing 10 p. p. m. Solutions of the ions alone, and mixtures of zirconium and the respective ions were used. Suitable blanks were used in all cases. Interference was noted if the ions gave a precipitate in the absence of zirconium and if precipitation was prevented or the character of the precipitate altered in the mixtures of the ions and zirconium. The limiting concentrations given in Table I are for a zirconium concentration of 10 p. p. m. At higher zirconium concentrations they are somewhat greater.

Phosphate and sulfate prevent the formation of the precipitate. Fluoride must be absent. In the case of aluminum, beryllium, chromium, ferric, thorium, and titanium ions, mixtures consisting of zirconium and the respective ions were precipitated as the hydroxides, the precipitates were filtered off and dissolved in nitric acid, and the interference was determined under conditions comparable to the preceding interference studies. The results were comparable to those listed in Table I.

Sensitivity

In the absence of interfering ions, the minimum concentration of zirconium that may be detected is 2 p. p. m. About 5 minutes of continuous shaking are required and a blank must be used. The reaction rate is slow at 5 p. p. m. A zirconium concentration of not less than 10 p. p. m. (0.5 microgram in 0.05 ml.) is recommended.

Colorimetric Determination of Cobalt with Terpyridyl

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IN STUDYING the terpyridyl method for iron (*2*), interference by cobalt was found to result from formation of an orange, complex ion of cobalt and terpyridyl. The constitution of the complex, according to Morgan and Burstall (*1*), is analogous to that of the ferrous complex in which two molecules of the triamine are held by coordinate forces around the octahedral structure of the metal. The two molecules of the base are believed to lie in perpendicular planes determined by the nitrogen atoms.

2,2′-Bipyridyl and 1,10-phenanthroline contain the same iron-specific cyclic N—C—C—N group and both are excellent reagents for iron, but neither gives sufficient color with cobalt to be of interest. 2-Pyridylhydrazine also gives colors with cobalt and iron but is not a satisfactory reagent for either.

The proposed method is simple and direct, requiring only the addition of terpyridyl reagent to a solution of cobaltous ion and measurement of the color which immediately develops.

Experimental Work

SOLUTIONS AND APPARATUS. The color-forming reagent was a 0.1 per cent aqueous solution of terpyridyl (2,6-di-2′-pyridylpyridine) hydrochloride calculated on the basis of the free triamine.

Standard solutions of cobalt nitrate containing 0.5 and 0.05 mg. cobalt per ml. were prepared by dissolving the desired amount of the hexahydrate of reagent grade in redistilled water and making appropriate dilutions.

Adjustments of pH were made with 6 *N* solutions of ammonium hydroxide and hydrochloric acid and buffering was accomplished with small quantities of a 20 per cent ammonium acetate solution.

Standard solutions of the anions studied were prepared from the alkali metal salts in most cases. Nitrates, chlorides, and sulfates were used for the cations. Each solution contained 10 mg. of the ion in question per ml.

Transmittancy measurements were made with a General Electric recording spectrophotometer set for a spectral band width of 10 mμ and pH values were measured with a glass electrode meter.

COLOR REACTION. Three to 5 ml. of the reagent solution should be used for 0.25 mg. of cobalt, or 10 p. p. m. in 25 ml. The slight increase in color intensity produced by doubling the quantity of reagent is not significant. Approximately the same amount of reagent is required for 0.25 mg. of iron. The hue is orange and absorption maxima occur at 445 and 505 mμ. A weak band at about 553 mμ can be isolated with an instrument operating on a spectral band width of 5 mμ or less. The molecular extinction coefficient at 505 mμ is 1360.

EFFECT OF pH. An exceptionally wide variation in pH is permissible, no effect on the intensity or hue resulting from changes between 2 and 10. This range is even wider than that permissible in the determination of iron with terpyridyl.

EFFECT OF COBALT CONCENTRATION. Beer's law is valid for cobalt concentrations between 0.5 and 50 p. p. m., the range most suitable for determinations with a 1-cm. transmission cell. Transmission curves for various concentrations are shown in Figure 1.

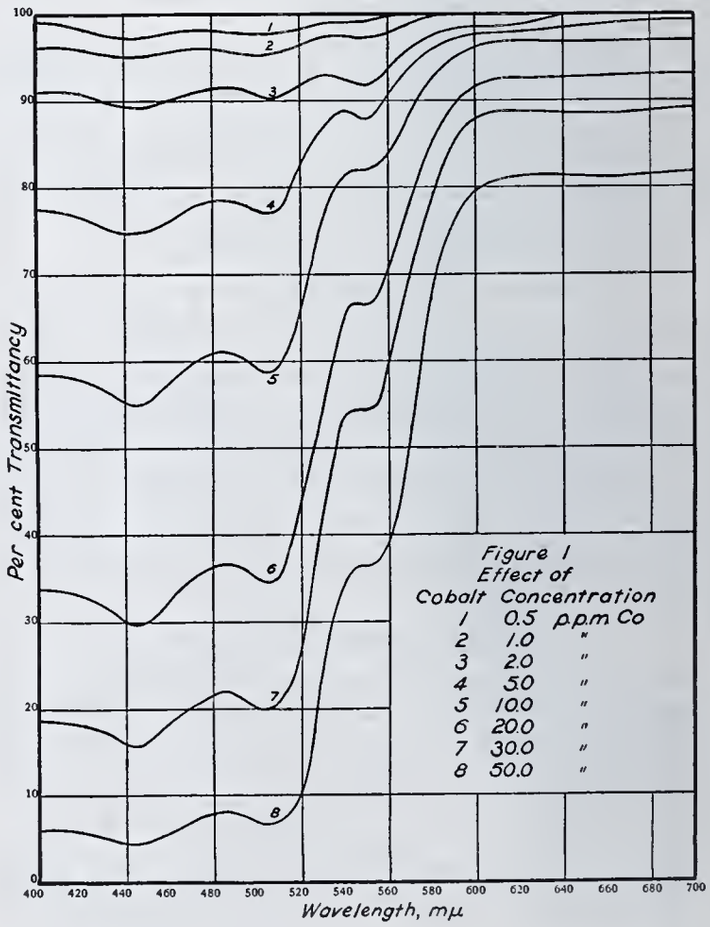
STABILITY OF COLOR. The cobalt complex contains an excess of 1 electron over the number required for maximum stability. Fading occurs within 24 hours at pH 3, necessitating the use of freshly prepared standards if the color measurements are to be made visually. A slight improvement was observed at pH 9, although the cobalt complex appears to be much less stable than the corresponding ferrous complex in any case. Transmission values for the solutions represented in Figure 1 increased 2 to 3 per cent in 24 hours.

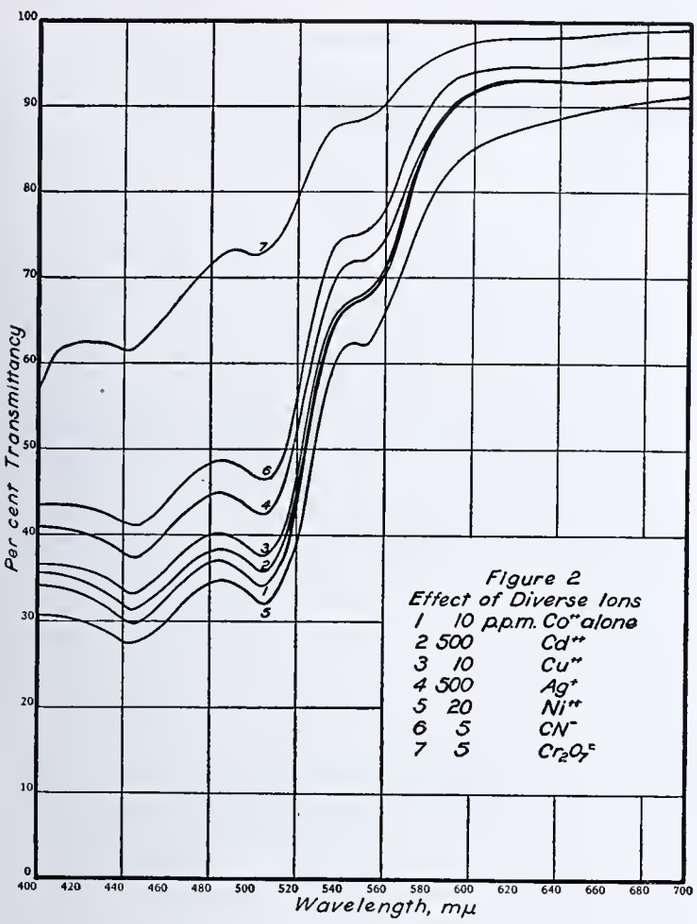
EFFECT OF DIVERSE IONS. In determining the extent of interference by diverse ions, the following procedure was adopted:

The desired volume of solution containing the ion under consideration was added to a 25-ml. volumetric flask containing 0.25 mg. of cobalt measured as cobalt nitrate solution. After addition of 10 ml. of water and 3 ml. of reagent, the solution was diluted to the mark and the transmittancy curve run, using 2-cm. transmission cells with water in the blank cell.

The following ions may be present in concentrations exceeding 500 p. p. m. without causing more than 2 per cent error in the determination of 10 p. p. m. of cobalt: aluminum, ammonium, barium, beryllium, calcium, lead, lithium, magnesium, manganese, potassium, sodium, strontium, thorium, acetate, arsenate, bromide, carbonate, chloride, citrate, fluoride, iodide, nitrate, nitrite, orthophosphate, oxalate, pyrophosphate, silicate, sulfate, sulfite, tetraborate, thiocyanate, and thiosulfate.

The ultimate effect of the interfering ions is summarized in Table I, which shows the approximate limiting concentrations permissible for the determination of 10 p. p. m. of cobalt with an error of not more than 2 per cent. Transmission curves for solutions containing several interfering ions are shown in Figure 2. Iron, cyanide, and dichromate must be absent. Antimony, bismuth, and tin precipitate under the conditions recommended.





Silver interferes slightly and no advantage can be gained by converting it to the ammonia complex. Copper, zinc, and cadmium, if present in sufficiently high concentrations, give low results for cobalt, apparently by complex formation with the reagent.

Titanium, zirconium, and cerium interfere somewhat. Reduction of ceric ion with hydroxylamine reduces substantially the interference of cerium. Uranyl ion, although colored, may be present up to 100 p. p. m.

Of the metals studied, chromium, nickel, copper, and iron cause the greatest difficulty. Attempts to eliminate iron interference by formation of ferric citrate or phosphate were unsuccessful.

Salts of most mineral acids do not affect the color reaction. Certain ions which affect the iron-terpyridyl reaction profoundly, such as carbonate, silicate, and phosphates, do not produce any measurable effect on the cobalt reaction.

Interference by dichromate, cyanide, and vanadate is sufficient to require separations. Five p. p. m. of dichromate caused an error of 70 per cent.

Discussion

Terpyridyl is an excellent reagent for iron according to the characteristics by which colorimetric methods are usually appraised. While the reagent is not so sensitive for cobalt as for iron, it can be used over a considerably wider range of concentrations in the determination of cobalt. The liberal tolerance in control of pH is significant. Most methods employing the organic reagents require considerable care in this respect.

One objection to the proposed method is the instability of the colored complex. Standards for visual comparison should be prepared for each day's work. This is only a minor consideration in view of the increasing use of photoelectric instruments. More serious is the interference of iron, copper, and nickel. Presence of these metals requires separations.

Nitroso R salt, commonly recommended, gives considerably more color with moderate amounts of cobalt (1 to 5 p. p. m.) than does terpyridyl, but for concentrations lower than 0.1 to 0.5 p. p. m. it is of little value because of the yellow color of the reagent itself. The advantage of sensitivity is thus confined to concentrations within the range susceptible to measurement with terpyridyl. Nitroso R salt also requires more care in control of pH and the color appears to be no more stable than that obtained with terpyridyl. Terpyridyl is not available commercially.

Table I. Effect of Diverse Ions

Ion	Added as	Present	Error	Amount Permissible
		P. p. m.	%	*P. p. m.*
Ag^+	$AgNO_3$	100	2	100
Cd^{++}	$Cd(NO_3)_2$	500	4	250
Ce^{++++}	$Ce(NH_4)_4(SO_4)_4$	200	4	100
Cu^{++}	$Cu(NO_3)_2$	5	0	5
Cr^{+++}	$Cr_2(SO_4)_3$	20	3	15
Ni^{++}	$Ni(NO_3)_2$	10	3	5
Ti^{++++}	$Ti(SO_4)_2$	75	3	50
UO_2^{++}	$UO_2(C_2H_3O_2)_2$	100	1	100
Zn^{++}	$Zn(NO_3)_2$	20	2	20
Zr^{++++}	$Zr(NO_3)_4$	100	2	100
AsO_3^{---}(As)	Na_3AsO_3	500	4	300
MoO_4^{--}	$(NH_4)_2MoO_4$	100	5	50
VO_3^-	KVO_3	20	4	10
WO_4^{--}	Na_2WO_4	100	0	100

Recommended Procedure

Treatment of Sample. Dissolve the sample by appropriate means and remove any interfering constituents in accordance with the tolerances listed in Table I. Adjust the pH to 2 to 10. (Twenty per cent ammonium acetate solution, 6 *N* ammonium hydroxide, and 6 *N* hydrochloric acid may be used.) Dilute the solution in a volumetric flask so that each 100 ml. contains 2 to 5 mg. of cobalt.

Measurement of Desired Constituent. Withdraw a 25-ml. aliquot of the sample solution, add 5 ml. of 0.1 per cent terpyridyl solution, dilute to 50 ml., and mix well. (Dissolve the terpyridyl in the least possible amount of 6 *N* hydrochloric acid and dilute to the proper volume with iron-free water.) Measure or compare the color by any of the usual means. (Standards for visual work should be prepared daily.) A blue-green filter such as Corning No. 428 and a 2-cm. transmission cell are recommended for photometric measurement.

Summary

A new colorimetric method for the determination of cobalt is described. Variations in pH between 2 and 10 do not affect the colored complex and Beer's law is valid for cobalt concentrations from 0.5 to 50 p. p. m. This is the optimum range for determinations with a 1-cm. transmission cell. Interference by most of the common metals except copper, nickel, and iron is not serious. Cyanide and dichromate should be absent. Because of the limited stability of the color, fresh standards for visual work should be prepared daily.

Acknowledgment

G. Stafford Whitby of the Chemical Research Laboratory, Teddington, Middlesex, England, very kindly furnished the terpyridyl.

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Presented before the Division of Analytical and Micro Chemistry at the 104th Meeting of the American Chemical Society, Buffalo, N. Y. Abstracted from a thesis presented by M. L. Moss to the Graduate School of Purdue University in partial fulfillment of the requirements for the degree of doctor of philosophy, May, 1942.

The Microvol

Apparatus for Measuring Small Volumes of Gas

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A SIMPLE and useful device for measuring very small volumes of condensable gas in a vacuum apparatus is the microvol. Volumes of the order of 0.05 cu. mm. can be measured within 10 per cent, the accuracy improving rapidly with increase in sample size. A single such device can readily be constructed to measure a complete range of volumes from 0.01 cu. mm. up to several milliliters. A microvol has been found very satisfactory in the analysis of the soil atmosphere for substances present in concentrations of less than one part per million, and should be generally applicable in microanalysis of gases.

As shown in Figure 1, the microvol is somewhat similar in construction to a McLeod gage, with the essential added feature of an inner-sealed bulb, *F*, opening to the outside. In use, the microvol is connected to the vacuum system at *E* through a mercury valve or stopcock. The apparatus is thoroughly evacuated, the gas to be measured being contained in a storage tube or held condensed in a trap. Liquid nitrogen is poured into *F* through *G*. The microvol is then opened to the tube containing the gas, which is allowed to expand into the closed vacuum system and condense on the outer walls of the cooled bulb. When condensation is complete, as indicated by a McLeod gage connected to the system, air is admitted to the mercury reservoir, *N*, through *K* and stopcock *M*, to force the mercury through *J* to level *I*, where it closes off the microvol bulb from the rest of the system. The entire gas sample to be measured is thus trapped.

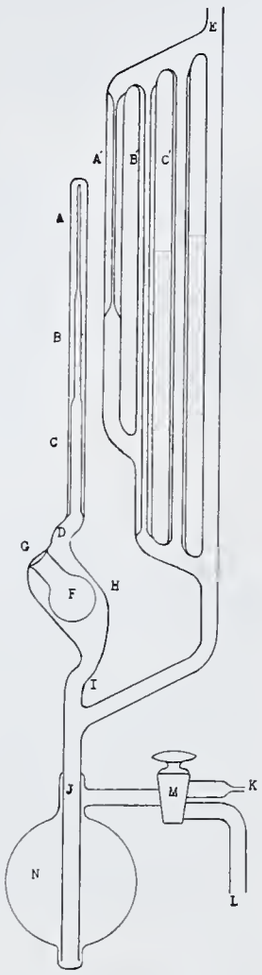

FIGURE 1

The liquid nitrogen is simply removed by allowing the mercury to touch bulb *F*, causing the nitrogen to boil out in a single spurt. The bulb is then warmed to room temperature, with the hand, by contact through the mercury, or by filling with warm water. After the mercury has been drawn down to *I* again by exerting a rough vacuum at *L*, the evaporated gas is slowly compressed into the calibrated capillary system *A*, *B*, *C* by raising the mercury, and measured at any volume at which it exerts a readily measurable pressure.

The microvol is constructed entirely of Pyrex. For measuring volumes from 0.05 cu. mm. up to 0.5 ml., the following approximate dimensions have been found satisfactory for the measuring capillary: *A*, *B*, and *C* are each about 10 cm. long, and 0.4, 3, and 6 cu. mm. per mm., respectively. Capillaries *A'*, *B'*, and *C'* are of the same stock as *A*, *B*, and *C*, since they are for use as manometers in reading the pressure of gas in the calibrated tube. Bulb *F* is 20 to 30 mm. in diameter, and opening *G* is about 10 mm. in diameter. Bulb *H* need be only slightly larger than *F*; a diameter of 35 mm. is sufficient. The calibrated capillary is joined to the top of *H* through a short piece of 8-mm. tubing, *D*. The mercury reservoir, *N*, may be constructed from a 200-ml. flask.

The measuring capillary must be carefully calibrated before sealing to the apparatus. In calibrating the joints, it is convenient to make a permanent mark on the uniform-bore capillary, close to the joint on each side. It is then only necessary to determine the total volume between marks, as the gas volume can be read either above or below each joint with ease. There will be no discontinuities in measurable volumes. Spare capillaries of the same stock can conveniently be calibrated and kept in stock for emergency repairs.

The microvol has the following definite advantages over a McLeod gage used for the same purpose: The entire gas sample, rather than a part only, is directly measured, so that the microvol is more sensitive. The pressure-volume products can be checked very simply by readings at widely different and quickly adjusted mercury levels. A much larger range of volumes can be measured in the same apparatus.

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Iodometric Semimicroprocedure for Determination of Arsenic in Sodium Cacodylate and Cacodylic Acid

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IN THE semimicromethod for the determination of arsenic in organic compounds described in a previous paper (*4*), the samples were decomposed with concentrated sulfuric and nitric acids. However, this method of decomposition is not applicable to cacodylic acid and its sodium salt. Attempts were made to decompose the cacodylate with sulfuric and nitric acids, but when the reduction with hypophosphite was carried out a yellow precipitate formed which volatilized upon heating. Upon further investigation, the most satisfactory method of decomposition for the cacodylate was found to be somewhat similar to the one used for the decomposition of minerals (*2*). This consists of heating the sample with potassium acid sulfate in the presence of small amounts of concentrated sulfuric acid. Specimens of neoarsphenamine were subjected to this treatment without successful decomposition. Therefore the method is recommended for sodium cacodylate and cacodylic acid only.

APPARATUS. The all-glass apparatus recommended for use in the determination of mercury (*3*) and arsenic (*4*) in organic com-

Table I. Determination of Arsenic in Sodium Cacodylate and Cacodylic Acid

Compound	Arsenic Found: Semimicro-method	Arsenic Found: Distillation method[a]	Arsenic Calculated
	%	%	%
Sodium cacodylate	46.63	46.71	
	46.65	46.55	
	46.65	46.85	
	Av. 46.64	46.70	46.83
Cacodylic acid	54.30	...	
	54.38	...	
	Av. 54.34	...	54.31

[a] Sample was decomposed in distillation flask with acid sulfate and sulfuric acid and analysis completed according to procedure of Association of Official Agricultural Chemists (*1*).

pounds is suitable for the analysis of cacodylic acid and sodium cacodylate.

Weigh the dried sample (of size to yield approximately 15 mg. of arsenic) on a piece of cigaret paper or in other convenient manner and transfer to the flask. Introduce into the flask 5 to 6 grams of potassium acid sulfate and 0.5 ml. of concentrated sulfuric acid, attach the condenser, and heat gently until all the potassium acid sulfate is melted. Continue heating vigorously until no further signs of carbon remain, allow to cool, and wash the condenser and walls of the flask with water. Dilute to a volume of approximately 20 ml., insert the glass stopper, and shake until the fused mass is dissolved. Add 3 grams of sodium hypophosphite ($NaH_2PO_2.H_2O$) and follow the original procedure (*4*).

The results in Table I show the method to be satisfactory.

An investigation is being carried out in applying the method to the determination of arsenic in arsenic-containing insecticides, such as calcium, magnesium, lead, and copper arsenates.

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Microbiological and Chemical Assay of Nicotinic Acid in B Complex Products

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THE important role of nicotinic acid (niacin) in human nutrition has stimulated the search for a method of assay which would possess the specificity of the biological assay with dogs and yet afford greater accuracy, speed, and economy. A number of chemical procedures based on the König cyanogen bromide reaction and several microbiological methods have been reported to meet these requirements. The dog method has not been used extensively for quantitative tests, so that comparative data are limited. The recovery experiments and duplication studies in connection with the various rapid methods are subject to limitations as criteria of the reliability of these procedures. It is believed that further comparative assay data from procedures as widely different as the chemical and microbiological tests will provide useful evidence as to their applicability. In the present studies tests have been made on various natural sources of the B complex. The chemical method as modified by Jones (*4*) and the Snell and Wright (*8*) microbiological method were used. Microbiological assays were made by R. D. Greene and A. Black, and chemical assays by F. O. Howland.

Methods

Microbiological. The procedure is essentially that of Snell and Wright (*8*). However, in the preparation of the hydrolyzed casein supplement, the optional charcoal treatment has been used as a regular procedure. In performing this step the authors treat a solution of hydrolyzed casein of pH 3.5, containing about 25 mg. of solids per ml., with 2.5 mg. of Darco G-60 per ml. Recently Isbell (*3*) has reported poor growth with charcoal-treated casein hydrolyzate, due to *p*-aminobenzoic acid deficiency. The authors' hydrolyzate, treated at greater dilution with charcoal, supported optimum growth. Although *p*-aminobenzoic acid was not a limiting factor in their tests, its routine addition seems a desirable safeguard against variations in the casein supplement.

For the preparation of inoculum they have used a dilution of 1 to 25 rather than 1 to 10. For incubation of cultures they have used temperatures of 30° C. and later 37° C. (*9*) with equal success.

In supplying the biotin requirement of the organism they have used the supplement prepared from egg yolk and later pure biotin with equal results. In their experience the free pure biotin at a level of 0.001 microgram per 10 ml. of medium was

Table I. Recovery of Pure Nicotinic Acid in the Presence of Wheat Germ Extract

Added	Found	Recovered	Recovery
Micrograms of nicotinic acid per 10 ml. of medium[a]			%
0	0.096	...	...
0.025	0.122	0.026	104
0.050	0.152	0.056	112
0.075	0.174	0.078	104
0.100	0.203	0.107	107
0.150	0.264	0.168	112
0.200	0.324	0.228	114

[a] Medium according to Snell and Wright +1.4 mg. of wheat germ extract per 10 ml.

Table II. Effect of Acidic Treatment of Samples on Microbiological Assay

Sample No.	Description	Nicotinic Acid: Aqueous	Nicotinic Acid: Acidic[a]
		Mg./g.	*Mg./g.*
2463	Yeast	0.214	0.231
2488	Yeast	0.603	0.599
2514	Yeast	0.218	0.229
2522	Yeast	0.342	0.371
2540	Yeast	0.560	0.488
2560	Yeast	0.506	0.512
2561	Yeast	0.194	0.216
2294	Yeast extract	1.80	1.85
2541	Yeast extract	3.04	2.83
2542	Liver extract	0.508	0.462
2287	Rice bran concentrate	1.64	2.15
2426	Rice bran concentrate	1.18	1.53
2485	Rice bran concentrate	0.97	1.33
2523	Rice bran concentrate	1.02	1.42
2459	Wheat germ extract	0.049	0.069
	Flour, patent	0.0069	0.0095
	Flour, whole wheat	0.0293	0.0362
	Yellow corn, ground	0.0156	0.0194
	Rolled oats	0.0051	0.0057
	Whole milk powder	0.0049	0.0051

[a] Autoclaving for 15 minutes at 15 pounds with 25 volumes of 2 *N* HCl or 2 *N* H_2SO_4.

Table III. Microbiological and Chemical Assays for Nicotinic Acid

Sample No.	Microbiological[a] No. of tests[b]	Range Mg./g.	Av. Mg./g.	Chemical No. of tests[b]	Range Mg./g.	Av. Mg./g.
			Rice Bran Sirup			
15,037	2	1.69–1.74	1.71	1	.....	1.61
13,917	2	1.41–1.42	1.41	1	.....	1.24
12,727	2	1.47–1.47	1.47	1	.....	1.24
9,279	2	1.78–1.79	1.78	2	1.78–1.82	1.80
7,922	2	1.63–1.69	1.66	1	.....	2.10
7,594	2	1.67–1.72	1.69	1	.....	1.50
7,280	2	1.65–1.73	1.69	1	.....	1.90
6,093	2	2.08–2.21	2.14	1	.....	2.20
5,538	2	1.88–1.91	1.89	1	.....	1.80
			Dried Yeast			
13,917	2	0.23–0.23	0.23	1	.....	0.36
12,009	6	0.21–0.25	0.23	3	0.17–0.24	0.20
11,322	4	0.34–0.43	0.38	2	0.32–0.35	0.33
10,811	4	0.43–0.46	0.44	2	0.36–0.40	0.38
9,925	2	0.34–0.36	0.35	2	0.37–0.42	0.39
6,532	2	0.54–0.55	0.54	1	.....	0.44
12,874	4	0.52–0.65	0.59	2	0.54–0.76	0.65
11,724	4	0.54–0.62	0.57	4	0.54–0.74	0.60
11,725	2	0.54–0.58	0.56	1	.....	0.39
12,875	4	0.58–0.62	0.60	3	0.42–0.57	0.50
			Yeast Extract			
14,236	2	1.63–1.75	1.69	1	.....	2.0
7,633	5	1.79–1.96	1.87	2	1.7–2.5	2.1
6,347	4	1.80–2.22	1.98	2	2.0–2.1	2.05
6,000	2	2.00–2.23	2.12	1	.....	1.8
5,514	2	1.91–1.95	1.93	1	.....	1.8
5,248	2	1.98–2.15	2.06	2	2.1–2.3	2.2
4,871	2	1.91–2.05	1.98	1	.....	1.8
			Wheat Germ			
7,991	2	0.067–0.071	0.069	2	0.066–0.071	0.068
7,646	2	0.071–0.071	0.071	2	0.060–0.070	0.065
11,716	2	0.074–0.079	0.076	3	0.064–0.089	0.078
			Malted Wheat Germ Extract			
9,414	4	0.096–0.111	0.102	1	.....	0.068
4,902	4	0.087–0.112	0.100	3	0.07–0.13	0.093
98,750	2	0.114–0.115	0.114	3	0.08–0.15	0.123
1,610	2	0.100–0.108	0.104	[a]	.....	...
15,351	2	0.103–0.104	0.103	[a]	.....	...
			Cane Molasses			
6A	2	0.0047–0.006	0.0053	1	.....	None
8A	2	0.0098–0.01	0.0099	2	None–0.04	...

[a] All other than yeast products prepared by acid treatment as in Table II.
[b] Independent tests.

more than sufficient for *Lactobacillus arabinosus 17-5*, whereas the methyl ester was almost inactive. Shull, Hutchings, and Peterson (*7*) have reported that the methyl ester is not utilized by *Lactobacillus casei* ε.

Chemical. The chemical assays of nicotinic acid were carried out by a modified cyanogen bromide method which has been fully described by Jones (*4*).

Factors Affecting Microbiological Assay

Specificity of Response. In order to test the sufficiency of the basal medium the authors added extracts of rice bran, yeast, and liver in amounts judged by assay to be equivalent to the levels of nicotinic acid in the standard series, up to optimum. The titration curves produced from the addition of these supplements to the basal medium followed the standard curve very closely and indicate that results based on the usual assay range involve negligible stimulation due to substances other than nicotinic acid. This question has been approached in a different way by determining the recovery of varying increments of pure nicotinic acid in the presence of a constant quantity of a wheat germ extract, a product relatively low in nicotinic acid. The recovery data from this experiment, shown in Table I, confirm the results obtained from the addition of the other B complex supplements.

Preparation of Samples for Assay. Several of the earlier reports on both chemical (*5*, *6*) and microbiological (*8*, *11*) assays of cereals indicated that the results were dependent to a marked degree on how drastic were the conditions of extraction. Recently other reports (*1*, *2*, *10*) have gone into this matter more extensively, showing that cereal products, as distinguished from yeast or animal products, contain a water-soluble precursor of nicotinic acid which yields maximum values in the microbiological assay only after treatment with alkali or strong acid. The question has been raised as to whether this microbiologically inactive precursor of nicotinic acid is active for animals, and the consequent issue as to the available nicotinic acid of cereal foods and feedstuffs must await clarification by further research.

The authors' experiences with a variety of products of cereal origin, ranging from original grains to highly potent concentrates, have paralleled those of other investigators. In order to determine the conditions necessary for the preparation of samples for test, they have made comparative microbiological assays of a variety of products subjected to treatments ranging from aqueous to 4 *N* acid. As a result of these experiments, summarized in Table II, they have adopted the use of strong acid as a standard preparatory treatment for cereals, whereas other materials have been prepared by aqueous extraction.

Microbiological and Chemical Assays

The results of parallel microbiological and chemical assays of various cereal and yeast products are presented in Table III. For the most part the results from the two methods are in agreement and indicate that both these rapid and relatively inexpensive tests are reliable measures of nicotinic acid, although somewhat greater individual variations were observed in the chemical tests of materials of lower nicotinic acid content.

Summary

In agreement with recent reports it has been shown that preliminary acid treatment of cereals, as distinguished from other products, leads to higher microbiological assay values for nicotinic acid.

Comparative assays of various pharmaceutical B complex products for nicotinic acid by the Snell and Wright microbiological method and by the cyanogen bromide method as adapted by Jones have shown substantial agreement between the results from these widely different procedures and tend to establish the validity of both as measures of this factor. In the testing of the materials of lower nicotinic acid content in this series the microbiological method appeared to be somewhat more suitable.

Acknowledgment

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Spectrochemical Assay for Traces of Tungsten

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A spectrographic procedure which is extremely sensitive in the estimation of traces of tungsten has been developed. The efficiency of the method depends on the tungsten-collecting properties of hydrated alumina when precipitated by sodium bicarbonate in dilute tungsten solution. This collector is efficient in a solution containing any quantity of alkali salts, and with it 0.00002 per cent of tungsten in an ore or concentrate may be isolated and estimated spectrographically.

THE research laboratory of this company was recently faced with the problem of determining trace amounts of tungsten in a series of classifier sands. A spectrographic survey revealed the feasibility of using this instrument in the final evaluation, but a check of the common methods in use for the isolation of tungsten (*3*, *4*) showed these methods to give incomplete separation for trace amounts. An investigation into the possibility of modifying these procedures, or developing others, was thus indicated.

Assay Procedure

Weigh 10 grams of 200-mesh ore or concentrate into a 400-ml. beaker, wet the sample well with about 20 ml. of water, and add 100 ml. of hydrochloric acid. Cover, digest at 60° to 70° C. for about 1 hour, and then boil down to approximately 25 ml. Cool, add 25 ml. of hydrochloric acid and 10 ml. of nitric acid, and again boil to former volume. Add 20-ml. portions of nitric acid with successive boiling down until chemical action is complete. Then add 20-ml. portions of hydrochloric acid until all nitric acid is removed. Take nearly to dryness on a low-temperature hot plate, but do not bake.

Take up in 10 ml. of hydrochloric acid and 20 ml. of water and heat on a low-temperature hot plate until all iron goes into solution. Dilute to 200 ml. and heat to just below boiling. Stir in pulp of about half of a No. 41 Whatman filter paper (12.5-cm.) and continue digestion on plate with occasional stirring for 15 to 20 minutes.

Filter through 12.5-cm. No. 40 Whatman filter paper and wash free of iron with hot 2 per cent hydrochloric acid solution. Both the residue and filtrate are further treated to recover tungsten.

Residue. Ignite gently in a No. 1 porcelain crucible until all organic matter is oxidized, add about 10 grams of powdered fused sodium bisulfate, and mix with residue. Heat in muffle at low temperature until ebullition ceases and then at bright red for about 10 minutes. Cover, remove, and cool until fusion has solidified. Dissolve in about 50 ml. of boiling water and then pour in thin stream into 100 ml. of boiling 10 per cent sodium hydroxide solution, constantly stirred. Boil about 5 minutes, settle, and filter, washing well with hot water. Discard residue.

Filtrate. Boil down to about 100 ml., adding 10 per cent sodium hydroxide dropwise during the process until ferric hydroxide just fails to persist. Pour this boiling, nearly neutral solution in a thin stream into 150 ml. of vigorously stirred, boiling 20 per cent sodium hydroxide solution in a 1-liter beaker. Cover, place on pad on low-temperature hot plate, and continue boiling about 30 minutes. Remove, add about 500 ml. of boiling water, and allow to settle. Decant and again wash with same quantity of boiling water. Decant, dissolve the residue in the least possible hydrochloric acid, boil down as above, and again pour into boiling sodium hydroxide. Filter decantations through 18.5-cm. No. 1 Whatman filter paper, finally transferring the residue to the filter paper and washing well with hot water. Neutralize the filtrate with hydrochloric acid, evaporate, and combine this filtrate with that from the residue treatment.

To this combined sodium tungstate solution contained in a 1000-ml. beaker, and diluted to 700 to 800 ml., add 3 to 5 ml. of 0.1 per cent methyl orange and after bringing to the neutral point with hydrochloric acid, add 5 to 10 ml. of excess acid. To this solution pipet 10 ml. of aluminum solution, cover, and heat to boiling. Transfer to a medium-temperature hot plate and with constant boiling add saturated sodium bicarbonate solution from a dropping pipet until the hydrated alumina just begins to precipitate. Boil for about 5 minutes, add more methyl orange, and resume the addition of bicarbonate at about 25 to 30 drops per minute until the solution is definitely basic. Add 10 to 15 drops of excess bicarbonate and boil for 2 to 3 minutes. Settle, filter through 12.5-cm. No. 41 Whatman filter paper, and wash with hot water.

Transfer paper and residue to a porcelain crucible, ash in muffle at low temperature and then at 800° C. for 30 to 60 minutes, cool, and weigh the oxides. By means of this weighing, any alumina extracted from the sample, over and above that added, is accounted for. If the increase in alumina is greater than a few per cent, increase the observed value of the tungsten concentration by the same amount to obtain the correct value.

Crush the oxides, but do not grind finely, and weigh 30 mg. into a drilled electrode. Arc together with standards and estimate the intensity of the tungsten line 2896.45 Å.

Table I. Recovery of Tungsten

Sample			Recovery		
SiO_2	$FeCl_3$	Tungsten	Residue	Filtrate	Total
Grams	*Grams*	*Mg.*	*Mg.*	*Mg.*	*Mg.*
1.0	..	0.20	0.06	0.14	0.20
2.5	..	0.20	0.08	0.11	0.19
..	5.0	0.20	...	0.20	0.20
..	10.0	0.20	...	0.195	0.195
1.0	10.0	0.20	0.065	0.13	0.195
2.5	5.0	0.20	0.075	0.125	0.20

Equipment

A Hilger automatic Littrow-type large quartz spectrograph is used. The arc image is focused upon the slit by means of a Hilger spherical quartz condenser and the exposure is regulated by means of a rotating sector between the lens and slit. The arc source is a motor generator set regulated to deliver 15 to 17 amperes at about 150 volts across the arc and ballast resistance.

Eastman No. 33 antihalation plates, 10 × 25 cm. (4 × 10 inches), are used to photograph the spectrum and are subsequently developed for 6 minutes in D11 developer at 65° F. and fixed in F5 fixer for 20 minutes.

The crushed sample is weighed into ordinary spectrographic graphite electrodes 2.5 cm. (1 inch) long and 0.78 cm. ($^5/_{16}$ inch) in diameter, and uniformly drilled 0.47 cm. ($^3/_{16}$ inch) deep with a 0.39-cm. ($^5/_{32}$-inch) drill. The samples and standards are arced to give a spectrogram as called for in A. S. T. M. specifications for zinc analysis (*1*). The line densities are read on Bausch & Lomb viewing stands by means of B. & L. magnifiers.

Standard Tungsten Solution. Prepare a standard solution by dissolving 0.136 gram of tungstic acid in the least possible quantity of dilute sodium hydroxide solution and diluting to 1000 ml. with water. This solution contains 0.1 mg. of tungsten per milliliter.

Aluminum Solution. Chemically pure potassium aluminum sulfate is used to prepare a solution containing 10 grams per liter of aluminum. Weigh 168 grams of the hydrate, add 100 ml. of hydrochloric acid, and dilute to 1 liter. For use in the preparation of standards, check the aluminum content of this solution by following the precipitation procedure with sodium bicarbonate, as previously described, and adjusting if necessary.

Preparation of Standards

The standards arced consist of definite amounts of tungsten in an ignited alumina base. The tungsten content of these standards is expressed as a percentage of their aluminum content; thus the 0.1 per cent standard contains 0.1 mg. of tungsten per 100 mg. of aluminum. The tungsten solution for the standards is measured out as follows:

Pipet 2.0 ml. of standard tungsten solution (0.2 mg. of tungsten) into a 100-ml. volumetric flask and dilute to volume. Pipet 50 ml. of this solution (0.1 mg. of tungsten) into a 1-liter beaker containing 10 ml. of aluminum solution (100 mg. of aluminum). Dilute to 700 to 800 ml. and precipitate the hydrated alumina with sodium bicarbonate, as described above, filter, and ignite to obtain the 0.1 per cent tungsten standard. Then dilute the 50 ml. of tungsten solution remaining in the 100-ml. volumetric flask to volume, again pipet 50 ml. with 100 mg. of aluminum, and repeat the precipitation procedure to obtain the 0.05 per cent standard. Continue this pipetting and diluting of the tungsten solution until the 0.0016 per cent standard is obtained. This was the ultimate dilution discernible by means of the author's spectrograph.

Table II. Recovery of Tungsten

	Residue from Preliminary Acid Treatment of 10-Gram Sample		Filtrate from Preliminary Acid Treatment of 10-Gram Sample		
(1) Weight	(2) Tungsten recovered in first $NaHSO_4$ fusion	(3) Tungsten recovered refusing insoluble from (2)	(4) Tungsten recovered after first iron removal	(5) Tungsten recovered on retreating iron ppt. (4)	Total Tungsten Recovered
Gram	*Mg.*		*Mg.*	*Mg.*	*Mg.*
0.23	0.024	N. D.[a]	0.003	0.001	0.028
0.14	0.023	N. D.	0.004	N. D.	0.027
0.29	0.015	N. D.	0.010	N. D.	0.025
0.17	0.012	N. D.	0.008	0.002	0.022
0.63	0.006	N. D.	0.003	N. D.	0.009
0.47	0.008	N. D.	0.002	N. D.	0.010
0.67	0.006	N. D.	0.004	N. D.	0.010
0.32	0.006	N. D.	0.007	0.001	0.014

[a] None detected.

Development of Procedure

In the preliminary spectrographic investigation, using various carriers, several persistent and satisfactorily graded lines in the tungsten spectrum were observed—namely, at 2656.54, 2896.45, and 2946.98 Å. (*2*). The use of the spectrograph as a means of final evaluation thus appeared possible and it was on the basis of spectrographic estimation that the procedure outlined was developed.

The common methods in use for the determination of tungsten claimed no great completeness in the isolation of traces of the element, so a preliminary check of these procedures became essential.

Using a pure sodium tungstate solution, the procedure of Schoeller and Powell (*4*) was followed up to the ignition of the tungsten-tannin-cinchonine complex. The ash was then dissolved in ammonium hydroxide, the ammonia boiled off, a standard amount of copper as a carrier added, and the solution sulfated and fumed to dryness. The spectrogram of this solid was then compared with that of copper sulfate, to which the same amount of tungsten had been added directly. It was found that from a solution containing 0.20 mg. of tungsten, an average of only 0.03 mg. was recovered, while none was recovered from a solution containing 0.02 mg. This separation was thus useless as a preliminary for spectrographic estimation.

An effort was then made to isolate the tungsten as mercurous tungstate, precipitating this compound from a carbonate solution with an excess of mercurous nitrate as outlined by Hillebrand (*3*). This precipitate was ashed at a low temperature, taken up in ammonia, and arced with copper sulfate as before. Precipitating from tungsten solutions containing 0.20 mg. per liter and a pH range of 3 to 9, recoveries of 0.09 to 0.04 mg. were obtained. Thus the use of mercurous carbonate as a gathering agent, while more efficient than cinchonine and tannin, was still not quantitative.

The use of a suitable collector appeared to be the most promising possibility. Numerous collectors were tried and it was finally found that hydrated alumina, as precipitated from boiling solution with sodium bicarbonate, gave a quantitative collection of tungsten.

An estimate of the efficiency of the alumina collector was first obtained by collecting various small quantities of tungsten in alumina, filtering, and sulfating the residue. Other samples were prepared by precipitating tungsten-free alumina as before, filtering, adding the same quantities of tungsten as had been collected in the previous series, and sulfating the residue. In all cases the corresponding tungsten lines observed were of the same average density. However, because of the low aluminum content of the aluminum sulfate arced (and thus of tungsten, since in the standards the tungsten is a definite percentage of the aluminum), the tungsten lines could not be discerned below the 0.025 per cent tungsten standard. By using an aluminum compound of lower molecular weight, the increased aluminum and tungsten content of the sample arced results in greater sensitivity of the tungsten lines. With the ignited alumina base, as recommended, the sensitivity was increased so that the 0.0016 per cent tungsten standard could be observed.

Because of the possible danger of loss of tungsten as tungsten oxide at high temperatures, a series of tests was run on standards to determine if the alumina base permitted such loss. A quantity of the standard was ignited strongly at 1000° to 1100° C. for 2 hours and this sample compared with the original, which had been ignited at a moderate temperature (<800° C.). In all cases, the standards showed no change in line density.

In the application of this procedure to ore analysis, it was found necessary to extract tungsten from both the residue and filtrate after the initial acid treatment.

In Table I are listed some results obtained, using pure silica and ferric chloride. The combined extraction from both the residue and filtrate under the procedure outlined thus appears quantitative.

The tungsten distribution resulting from the preliminary acid treatment of a series of samples and the sensitiveness of the procedure outlined are shown in Table II. The samples are high in sulfides and contain 1.4 to 6.7 per cent of acid-insoluble matter.

All tungsten is recovered from the residue in one fusion. A small additional recovery is made in some cases on retreating the iron precipitate from the preliminary acid treatment filtrate. Thus two successive extractions are specified in the procedure.

In the procedure outlined for ores, various other elements are collected by the alumina and are readily observed in the spectrogram. Their collection is not necessarily quantitative. They include lead, arsenic, antimony, tin, zinc, bismuth, and silica, among others, and after ignition are weighed together with the alumina as Al_2O_3. Unless these elements form an unduly large percentage of the oxides weighed and affect the carrier characteristics of the alumina, they will not otherwise change the calculated tungsten content.

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A Fractional-Distillation Microapparatus

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ONE of the most important operations in organic chemical work is distillation. Several excellent working apparatus have been designed during the past 30 years for fractionating macroquantities of liquids under normal and reduced pressures, among which are those of Kubierschky (*4*), Friedrichs (*2*), Widmer (*8*), Midgley (*5*), Othmer (*6*), and Jantzen and Tiedcke (*3*). The latter, with its specially constructed receiver unit, is widely used as a standard apparatus for the separation of high molecular fatty acids in the form of their esters.

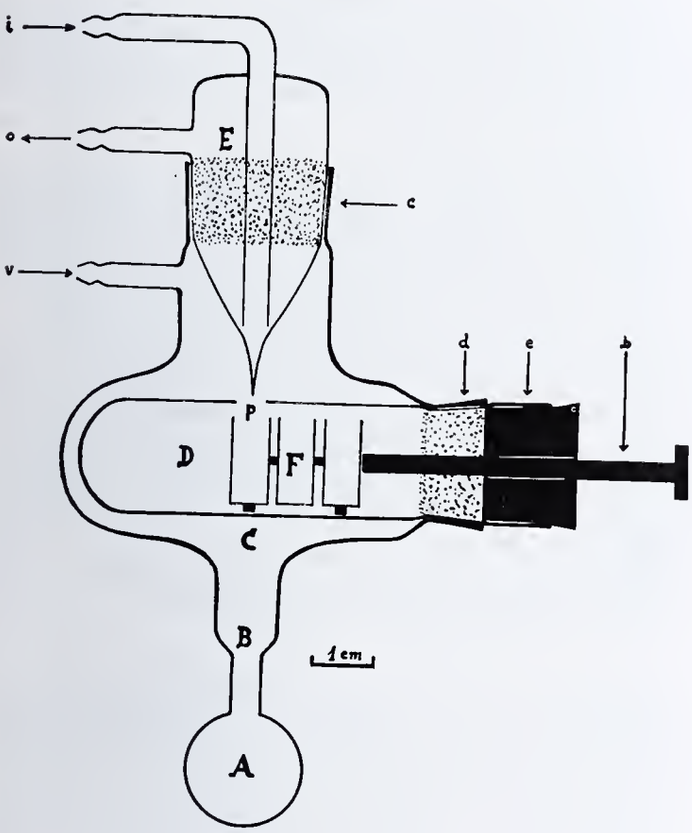

FIGURE 1. DIAGRAM OF APPARATUS

Microdistilling equipment, however, has not received the same concentrated attention, despite the fact that improvements in microchemical procedures generally have kept pace with the tremendous progress in the chemistry of vitamins, hormones, and other biologicals. This may partly be due to the fact that an accurate microthermometer has not been developed. As a result, microdistillations are used primarily to purify samples and not for sharp separation of fractions as in macrodistillations. In fact, much of the dissatisfaction with microdistillations often stems from attempts by operators to make quantitatively sharp fractionations with equipment (including that described herein) which is obviously limited to qualitative purifications. However, arbitrary fractions can be made as a first step to closer refractionation. Some microdistillation apparatus for making arbitrary fractions have been reported, noteworthy among others, those of Craig (*1*) and Shrader and Ritzer (*7*). The limitation of microdistilling equipment to purifications cannot be too strongly emphasized.

Because as much as 5 to 20 per cent of the initial sample in distillations, for all practical purposes, can be considered lost, owing to retention of liquid by the surfaces of the apparatus, it becomes obvious that microdistillation apparatus should be designed with minimum surface areas and minimum distance between the distilling flask and receiver. Only in this way can maximum recovery of sample and distillate be obtained. Unfortunately, some of the published designs do not meet this very important requirement.

In his widely diversified microchemical practice, the author repeatedly encountered need for improved microdistilling apparatus. It was apparent that suitable apparatus, to meet these needs and requirements, could not be built by the simple expedient of reducing the dimensions of efficient macrodistilling apparatus. Consequently a new apparatus was designed in which minimum distance between distilling flask and receiver was obtained by using an "inside receiver" and which also permitted collection of the arbitrary fractions without interruption of distillation.

Because of the size and shape of the new apparatus, exact calculations are either extremely difficult or impossible, and proper proportioning of the parts was made solely on a trial and error basis in various experimental models. Citing the dimension of these earlier models is without value. For most purposes, the dimensions of larger or smaller equipment to purify materials with boiling points falling between 60° and 300° C. can be made proportional to those of the unit here described. If departure must be made from the proportions given, a very elementary principle of distillation should be rigidly adhered to—i. e., the volume of the distilling flask plus the volume of the condenser chamber and connections should be substantially less than the volume which the sample will occupy when transformed into vapor at its boiling point. Unless this principle is observed, reflux action will prevent distillation, especially with high boilers or mixtures with high boiling components.

Apparatus

The apparatus (Figure 1) consists of the distilling flask *A* with a capacity of 3 to 5 ml. The lower section of neck *B* is 5 mm. in diameter; its upper section, 10 mm. in diameter, connects the flask with the chamber, *C*. This chamber has two openings; the one on top is 25 to 30 mm. in diameter and into it is fitted condenser *E* with a ground-glass joint at *c*. (Ⓢ 1/2 ground-glass joints, while not specified for the model which the author is currently using in his practice, will be used in future units.) The condenser is drawn out to a tip and is cooled by a stream of cold water which enters through the glass tube, *i*, filling the condenser and emerging at *o*. Since the effective condenser surface is small, tubes *i* and *o* are of a relatively large diameter (5 mm.) to permit very rapid replacement of cooling water. Tube *D*, 20 to 25 mm. in diameter, lies within *C* and is fitted with a ground-glass joint at *d*. This tube has a 5-mm. hole at *p* through which the distillate drops from the tip of the condenser into the receiver unit, *F*. This unit consists of 3 small beakers of 1-ml. capacity each, made of glass and attached to each other by fusion. It is placed in tube *D* through the opening which is closed during distillation with rubber stopper *e*. *D* has a slightly flattened floor to provide a better footing for the receiver. The two outer beakers of the receiver unit are fitted with glass buttons elevating the beakers 0.6 cm. (0.25 inch) above the flattened floor of *D* to insulate the beakers, so that reheating of the distillate is re-

tarded as much as possible when running high boiling point samples. The receiver is moved under the condenser tip by means of the glass rod, *b*, which passes through the bore of stopper *e*. (*b* is not fused to the receiver unit, as might appear from the picture. Adjustment of the receiver unit could perhaps be made easier at first by providing a glass loop on the unit to be engaged by a hook on the end of *b*.) Since the rod must fit tightly, it may be lubricated with glycerol if necessary. The apparatus is easily and quickly assembled and cleaned.

Operation is very simple. The liquid to be distilled is placed in *A* through the top opening by means of a pipet. For vacuum distillations, a porcelain chip is added to suppress bumping. However, even if bumping occurs, the collected distillate is not likely to be contaminated, since the receiver is almost entirely shielded from splashed or entrained liquid. This protective arrangement, which also prevents the rubber stopper from coming into close contact with the vapors, is one of the principal features of the design. Heat is applied according to requirements, by direct flame, a constant-temperature bath, or electric hot plate.

For best results heat should be slowly and carefully applied. All the distillations cited, including the acetone–ethyl alcohol mixtures, required a minimum of 15 minutes. Since microdistillations are usually run without temperature readings, a fair degree of temperature control is obtained by the thermometer of the bath or a calibrated rheostat in the hot-plate line. The apparatus may be used for distillation under normal or reduced pressures. Its efficiency has been tested with many liquids with boiling points from 60° to 300° C. as follows:

Three groups of liquids were selected for tests with boiling points under 120° for distillation at normal pressure, 120° to 200° for distillation at about 15 mm. pressure, and 200° to 300° for distillation at about 0.1 mm. For the first group, 3-cc. samples of acetone and ethyl alcohol mixtures were made in the ratios of 1 to 1, 1 to 2, and 1 to 3. On distillation of the 1 to 1 mixture, the first fraction of about 1 cc. was found to be pure acetone, as evidenced by refractive index and odor. The third fraction, also of about 1 cc., was found to be pure ethyl alcohol. Similar good results were obtained for the other ratios.

For the second group a mixture of benzaldehyde (boiling point 179°) and benzoyl chloride (boiling point 198°) was distilled at a pressure of about 15 mm. Carbon and hydrogen determinations showed the first and third fractions to be practically pure separations.

For the third group a mixture of lauric acid (boiling point 225°) and myristic acid (boiling point 250°) was distilled at 0.1 mm. and gave almost complete separation of these two fatty acids. Separate refractionation of the first and third fractions then yielded pure fatty acids as determined by elementary analyses.

The efficiency of separation of any distillation equipment is governed by many factors. The one factor which imposes greatest limitation on a microdistillation apparatus is that of the range of difference in boiling points of components in a mixture. In the equipment described, mixtures with components having only a 20° difference in boiling points are readily separated without interruption to collect the lower fraction. As the difference increases to about 40° continuous fractionation becomes increasingly difficult, and with a difference of over 50° fractions must be taken off separately. When continuous separation is attempted, the lower boiling component, depending on the vacuum used, will either be exhausted by the vacuum system, or condensed, reboil, and also be discharged by the vacuum system.

Thus for a mixture involving components with more than a 50° difference in boiling points, this apparatus can recover only one component continuously, though both may be recovered if the convenience of continuous operation is sacrificed. For materials with boiling points above 250°, fractionation becomes increasingly difficult because of bumping tendencies, larger losses due to surface wetting, and the tendency for refluxing to occur at higher distillation temperatures.

Microdistillation apparatus is intended primarily for purifications by means of distillation methods. Several practical cases encountered by the author in his practice illustrate its use.

A research residue of about 5 cc. was submitted for purification and confirming identification. The sample was thought to be quinoline (boiling point 238°) containing about 10 per cent of aniline (boiling point 184°). On distilling a 3-cc. sample, the last two beakers of the receiver (about 1 cc. each) contained pure quinoline as determined by elementary analysis. The fraction in the first beaker proved to be aniline.

Another research residue consisting of γ-picoline (boiling point 143°) containing about 20 per cent of α-picoline (boiling point 128°) was separated at 15 mm. and the last two beakers (1 cc. each) yielded pure γ-picoline checked by refractive index. Ten per cent of the 4-cc. sample distilled was lost.

In the above cases both components were recovered and identified. An example of wide differences in boiling points was presented by purification of a glycerol-water mixture submitted for identification of glycerol. Because a vacuum of 0.1 mm. was used, the water estimated to be about 10 per cent could not be collected, since at this pressure it passes into the vacuum system without condensing. Pure glycerol was obtained and checked by both refractive index and elementary analysis. Only 75 per cent of the glycerol was recovered. The comparatively high loss of 25 per cent was caused by heavier wetting films due to high viscosity.

While these examples generally indicate use of this microdistillation equipment, it is difficult to state precisely the exact limitations of any microdistillation apparatus for purification work. Each problem requires individual handling and successful use is largely dependent upon the skill and judgment of the operator.

Since the term "microquantities" is purely relative, samples smaller than 5 cc. are, in the author's conception from the standpoint of distillation, considered "micro" and are best distilled in the apparatus described. For samples larger than 5 cc. macro designs can be employed because in most instances losses of sample due to retention by glass surfaces are of no consequence. The apparatus with the dimensions described in this paper is suited to distilling 2- to 4-cc. samples. The same design properly proportioned can be made for any microquantity down to 0.5 cc. For still smaller samples, the apparatus of Craig (*1*) is recommended, which collects the distillate as an adhering drop on the indented tip of the condenser.

In the design of the apparatus described, the size of the receiver beakers is a critical factor for determining the other dimensions of the apparatus parts.

If, for example, 1 cc. of liquid is to be distilled and three arbitrary fractions are to be taken, the total capacity of the three receiver beakers need not be larger than 1 cc., thus making the volume of each beaker about 0.3 cc. Such a receiver unit can be constructed much smaller than a receiver unit of 3-cc. capacity, and hence tube *D*, chamber *C*, and condenser *E* can all be of smaller dimensions. It also follows that as the number of beakers in a receiver unit are increased, their individual capacity must be decreased in order to maintain their proper relation to tube *D*, location of hole *p*, and the condenser tip.

Since parts can be easily made in proportion, an apparatus of proper size can be made for any microquantity from 0.5 to 5 cc., making it for all practical purposes a truly universal microdistilling apparatus.

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Synthetic Rubber

A Spectroscopic Method for Analysis and Control

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INFRARED spectroscopy offers methods for the identification, analysis, and control of hydrocarbon mixtures, which have decided advantages over physicochemical methods now in use.

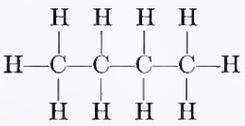
Ordinary chemical methods for the analysis of mixtures of hydrocarbons are long and tedious, and the trade has long resorted to the method of physical separation of the components by fractional distillation, with identification of the components by their boiling points. This is indeed an accurate method, but is time consuming. Even though the analysis can be accomplished in a matter of hours, many hundreds of gallons of product would be produced in a plant before an error in production could be caught in the laboratory. Therefore a method of analysis which would require at most a matter of minutes, and, in control, a matter of seconds, is highly desirable.

Another point for consideration is the fact that distillation requires samples in the liquid state. For the several hydrocarbons which are gases at normal temperatures, this requires condensation of the gases in order to obtain samples, and the usual difficulties of low-temperature distillations. The spectroscopic method, on the other hand, works equally well with gases or liquids, and requires only a very small fraction of the amount of material needed for distillation. Furthermore, this method is readily adaptable to use in the plant, since the spectrometer can be so placed as to allow the absorption cell to be connected into the production line. A portion of the product can be by-passed through the cell, thus obviating the necessity for transfer of samples to a separate laboratory. Lastly, the addition of a relay device permits automatic process control—a mechanical, continuous "watchman".

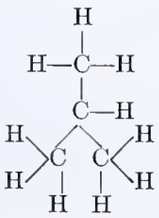
It has been pointed out many times in the literature (*1*, *7*, *8*) that the infrared spectrum of an organic compound is a unique property of that compound, and that, except in special instances, it retains that property on admixture with other compounds. These special instances are predictable, and are of no interest in discussing simple hydrocarbons. Thus, in general, in any particular hydrocarbon mixture, the concentration of a given component can be determined by infrared measurements, if at least one absorption band of this component can be found at a wave length for which the remainder of the mixture has negligible absorption. A determination of the per cent transmission at this wave length enables one to measure the amount of this component which is present.

That the problem of spectrochemical analysis by infrared is essentially simple may be readily seen by considering the various molecules of the components as mechanical systems. The origin of infrared spectra lies in the mechanical motions of the atoms of the molecules. (Actually, of course, the origin of infrared spectra lies in the periodic variation of the dipole moment of the molecules. However, this is a complicated function and a discussion is out of place here. It can be found in any standard text on spectroscopy.) Therefore, in discussing the mechanical analogy, we are approximating the actual spectroscopic situation, since each absorption frequency is related to a particular mechanical frequency. If we have several bodies tied together by suitable springs, and allow these masses to move freely, restrained only by the springs, they will perform certain motions with particular or characteristic frequencies. [The correlation between motions of gross bodies bound by steel springs and motions of atoms in molecules has been shown by Kettering, Shutts, and Andrews (*4*).] These frequencies can be made to vary by changing any of three factors—the masses involved, the strength of the springs, or the orientation of the masses.

Choosing a specific chemical compound as an example, let us take the molecule butane:



These carbon and hydrogen atoms will vibrate with certain frequencies which will give rise to a characteristic spectrum. If we simply rearrange this same number of atoms into another structure, we have isobutane:



From the mechanical analogy, it may be seen that this reorientation of the masses will give rise to characteristic frequencies different from those of *n*-butane.

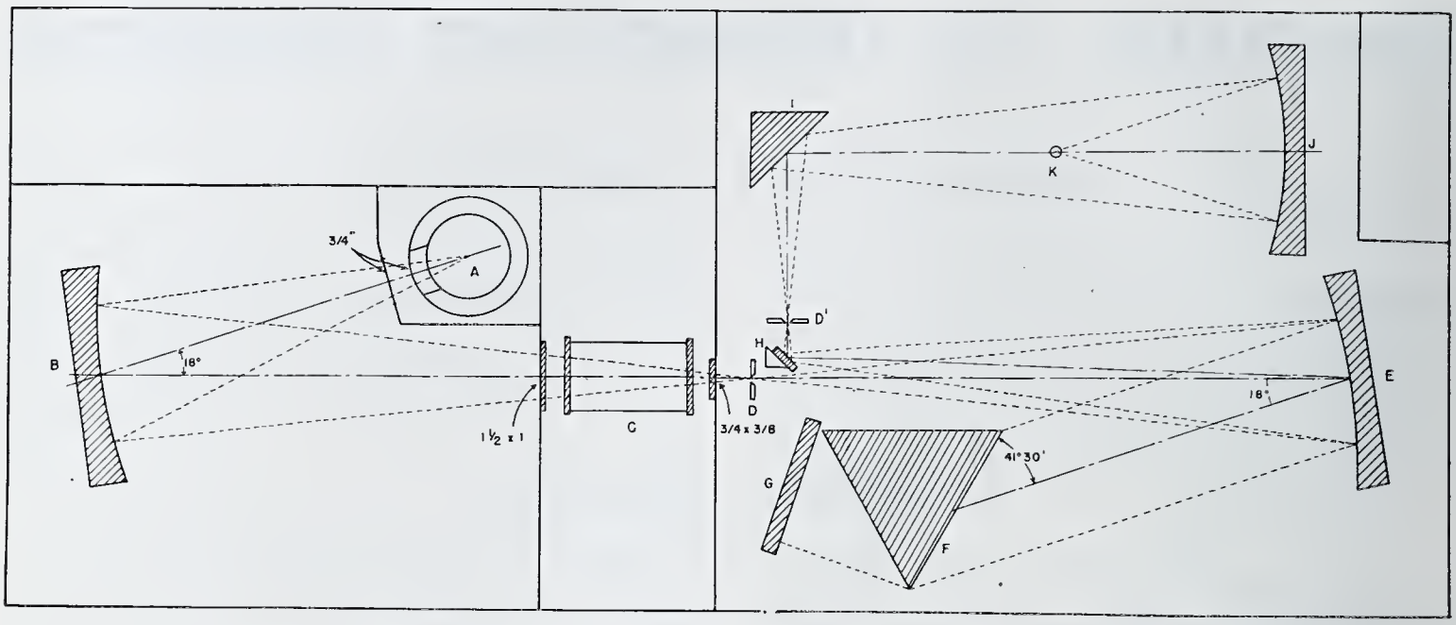


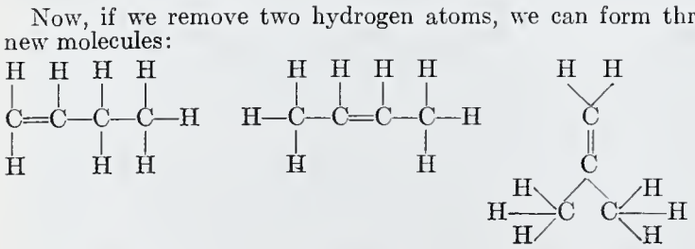
FIGURE 1. SCHEMATIC DESIGN OF A COMPACT, OPTICALLY POWERFUL INSTRUMENT FOR MAKING SPOT ANALYSES

Base, 10-inch channel iron
A. Source, Nernst glower

		Diameter, cm.	Focal length, cm.	Diopter
B.	Spherical mirror	9	10	10
E.	Spherical mirror	9	25	4
J.	Spherical mirror	9	7.14	14

C. Sample cell
D, D'. Slits
F. 60° salt prism, 6 cm. high, 7.5-cm. base
G. Plane mirror, 7 × 6 cm.
H. Plane mirror, 1 × 1.5 cm.
I. Prism, B. & L. 1.25-inch glass
K. Thermocouple

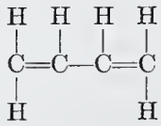
Now, if we remove two hydrogen atoms, we can form three new molecules:



Butene-1 Butene-2 Isobutylene

The decrease in mass caused by the removal of the hydrogen atoms can be neglected, as it is only 3 per cent of the total mass. Of importance, however, is the fact that we have double the strength of one of the "springs" holding the atoms together, and hence have a faster frequency of motion between two carbon atoms than occurs in either of the butanes. Furthermore, each of these new molecules is a mechanical system different from the other two.

Finally, by removal of two more hydrogen atoms from butene-1, we arrive at the molecule of prime interest, butadiene:



At first glance, it might be assumed that the frequency of the >C=C< spring here might be the same as in the previous molecules. Actually, however, another physical phenomenon appears, because there is a strong interaction between the motion of the carbon atoms, in this case roughly analogous to the interaction between two coupled pendulums. The organic chemist calls such molecules "conjugated" hydrocarbons or conjugated dienes, and knows that their chemical properties differ markedly from the monoolefins.

The foregoing molecules which have been chosen for purposes of illustration constitute the so-called C_4 fraction of petroleum distillations. Since they have different frequencies of motion, they give rise to characteristic infrared spectra, and so may be detected and measured quantitatively in hydrocarbon mixtures. In view of the fact, however, that the correlation between infrared absorption bands and mechanical motion holds in general, the application of this method of spectrochemical analysis is in no way limited to the cases herein cited. This method may be used in the cases of many rather subtle organic analyses, such as the differentiation of isomers, rates of oxidation, polymerization, etc.

It is the purpose of this paper to present the infrared spectra of certain compounds of particular interest in the manufacture of synthetic rubber, and to describe a method for analysis and for production control. The spectra of several synthetic rubbers are also shown.

Experimental

The apparatus used in this work has been described (*2*, *3*, *5*), together with the spectra of a large number of organic compounds and the analytical and identification techniques used.

The spectra shown in this article were obtained from an automatic recording, rock salt prism, Littrow spectrograph. The usual working range is from 3750 cm.$^{-1}$ (2.7μ) to 750 cm.$^{-1}$ (13μ). The average spectral slit width employed decreases from 15 cm.$^{-1}$ at 3000 cm.$^{-1}$ to 5 cm.$^{-1}$ at 800 cm.$^{-1}$.

Within experimental error, all measurements made on this instrument follow Beer's law, $I/I_0 = e^{-kcx}$. Here I/I_0 is the per cent transmission (or $1 - I/I_0$, the per cent absorption) at a particular frequency, k is an absorption constant of a compound at that frequency, c is the concentration of the compound, and x is the path length used. I/I_0 is the direct experimental result measured with a spectrograph and the accuracy with which it can be measured is determined by the characteristics of the instrument being used. Bearing this in mind, there are two pertinent considerations concerning the exponents kcx. First, except for a few special cases, at a given frequency I/I_0 remains constant so long as the product cx remains constant. Hence the concentration—(i. e., pressure in case of gas work)—or the cell length can

be chosen arbitrarily to suit experimental conditions so long as the other factor can be changed to give a suitable cx value. Second, if k and x are held constant, a consideration of the exponential graph for Beer's law shows that a small error in the measurement of I/I_0 represents different absolute errors in the measurement of the concentration, depending upon whether the concentration is high or low. Roughly speaking, the absolute concentration of a component can be measured more accurately when the concentration is low than when it is high. These points are elaborated below.

The spectra shown have been plotted between 2000 cm.$^{-1}$ and 750 cm.$^{-1}$, because this is generally the region from which the most information can be obtained. Results obtained outside of the region shown are discussed wherever they are of importance. These spectra are obtained from automatic recordings of the transmission through an empty and a sample-filled cell. These records are measured at given frequency intervals and the quotient of each pair of measurements is plotted as a graph of frequency in cm.$^{-1}$ *vs.* per cent transmission. Since there may be a change in experimental conditions between these two records and since there is no attempt to take account of scattered light, the intensity scale shown is of relative but not absolute importance. However, the frequency position of the bands is accurate within the experimental error of the instrument.

Automatic recording of cell-empty, cell-filled transmissions at a given frequency for "spot" analyses is chiefly of value in measuring a long series of similar samples. Moreover, the size of the authors' recording drum limits the amount of the cell-empty transmission energy which can be used. Hence all quantitative results given in this paper were obtained by visual reading of the galvanometer spot from an auxiliary lamp and scale system.

A description of the methods of infrared spectrochemical analysis is best achieved by taking a simple example in which it is desired to obtain the concentration of one component in a mixture of known materials. The spectra of all the pure compounds present in the mixture are compared and an absorption band is chosen which is unique to the particular component of interest. Transmission measurements at the frequency of the chosen band are made on a series of known standards in which the concentration of the one component is varied. A working calibration sheet is prepared by making a plot of concentration against these measured transmissions. Once the calibration data are obtained, any unknown may be analyzed by filling the absorption cell, measuring the per cent transmission on the instrument, and reading the answer off the work sheet—an operation requiring a very few minutes. By choosing a band unique to another component of the mixture and preparing a calibration work sheet at that frequency, two components may be measured successively. This process can be continued as long as these unique bands or combinations of such bands can be found. Working calibration sheets such as those described above have been obtained for various mixtures and are illustrated below.

There may be some objection that this method of analysis does not make allowance for the absorption of the empty cell or for the scattered light present in the instrument. It is true that corrections for these factors could be made and the working data could be plotted as a straight-line graph of log I/I_0 against concentration. Such corrections, however, are a function of the particular instrument used and would be of no value for analyses made on another instrument. Moreover, a working sheet on this basis would require additional calculations before it could be used. In actuality, the accuracy of any analysis depends ultimately upon the accuracy with which a per cent transmission can be measured, and the computational step from this measurement to concentration analysis should be as short as possible.

Because of the persistent requests for suggestions concerning infrared apparatus the authors have designed in this laboratory a small, very simple spectrometer with sufficient resolution and optical power to satisfy the demands of the problems at hand. This instrument was built by the Porocel Corporation and is now in very satisfactory service for hydrocarbon analyses at their plant.

A schematic diagram of the spectrometer is given in Figure 1. The light path through the instrument is shown in dotted line. The instrument has an aperture of f. 3.5 and the slits are adjustable to give the combination of energy and resolution desired for a given analysis. The design is a typical 60° rock salt prism Littrow mounting. In its present use, the absorption cell is introduced into the light path on a cell way and hand readings are made from a galvanometer and scale. If the instrument is to be used as a control relay in the plant, the absorption cell could be fixed in the light path and gas from a by-pass in the production line would be blown continuously through it. The introduction of a suitable device in the amplifying system would make possible the opening and closing of valves, variation in heat input in the process control, or the sounding of alarms. The instrument may be used for the analysis of liquid or solution samples merely by introducing a suitable absorption cell in the light path.

A photograph of the completed spectrometer is shown in Figure 2. The apparatus is shown from the operator's viewpoint with the galvanometer lamp and scale used in making analyses. The two glass leads to the absorption cell can be seen projecting above the instrument. Figure 3 shows the completed spectrometer with the covers removed and a foot rule laid on the base for size comparisons. This view is directly comparable with the schematic diagram of Figure 1, so that the component parts may be determined by corresponding positions on the base. The performance of this small spectrometer with respect to optical power, resolution, and reproducibility far exceeded expectations at the time it was designed.

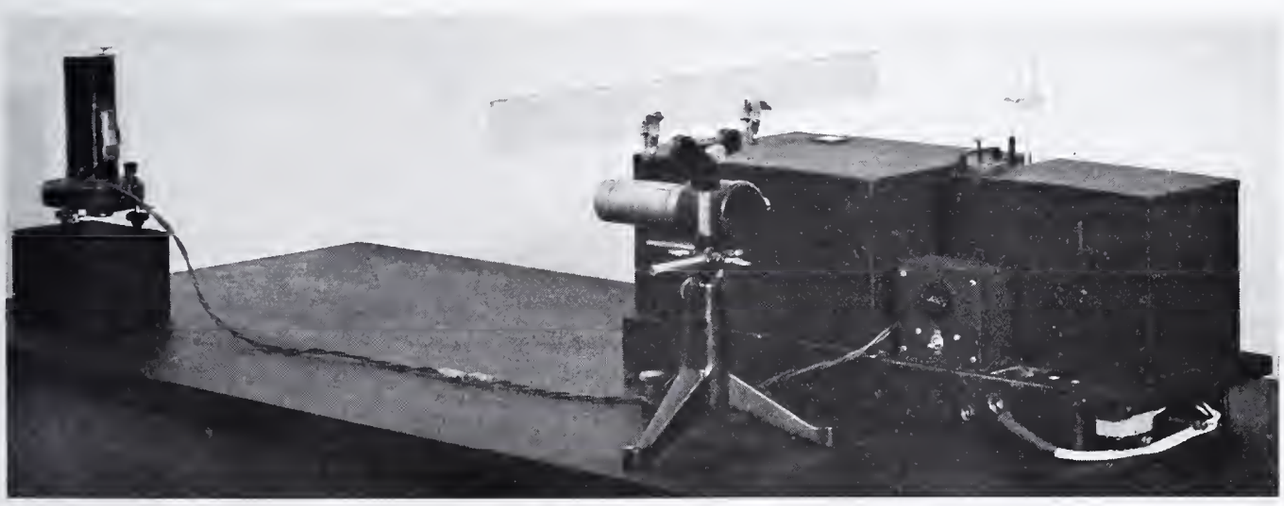


Figure 2. Small Infrared Spectrometer for Spot Analyses

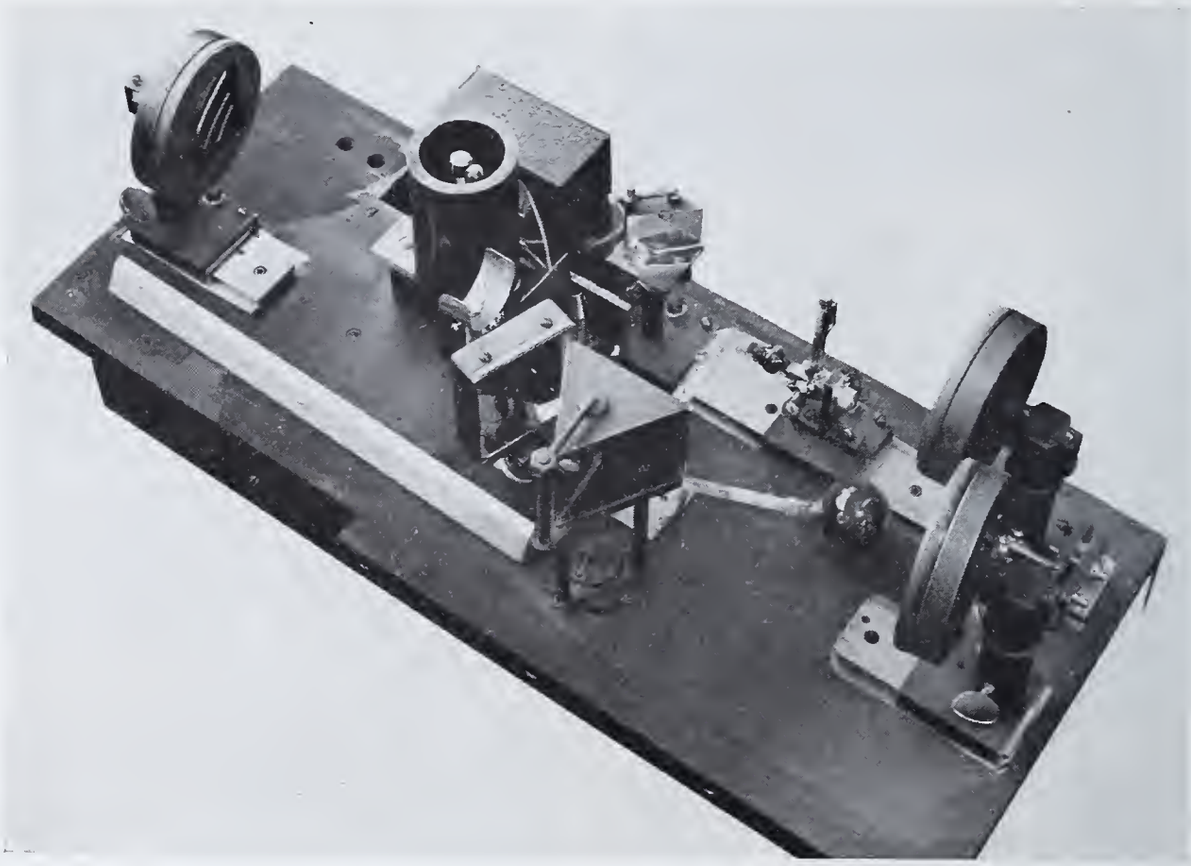


FIGURE 3. SMALL INFRARED SPECTROMETER WITH COVERS REMOVED

Discussion

An infrared spectrum may be divided roughly into two parts, a high-frequency and a low-frequency region, the division line being somewhere in the neighborhood of 1300 cm.$^{-1}$. In the high region, the observed absorption bands are likely to be caused essentially by a vibration of a specific pair or group of atoms within a molecule, such as O—H, C—H, N—H, C=O, C=C, aromatic rings, etc. In the low region, on the other hand, bands arise from vibrations in which all the atoms of the molecule take part. Upon comparing the spectra of an unknown and a known molecule, complete coincidence of bands both in position and intensity in the high region indicates that the same atomic groups are present, but does not necessarily permit the conclusion that the molecules are identical. On the other hand, since the lower frequency bands are more nearly characteristic of the molecule as a whole, a matching of their spectra throughout both regions would certainly show that the known and unknown molecules are identical. These characteristics, as well as their use in analytical work, are illustrated in the discussion below.

Figure 4 shows the spectra of *n*-butane, isobutane, and a mixture of 36 per cent *n*-butane, 60 per cent isobutane, and 4 per cent other gaseous hydrocarbons. Both spectra of the pure compounds have strong bands at 1450 cm.$^{-1}$, a general C—H group bending vibration. Again, both have a band around 1375 cm.$^{-1}$ which is characteristic of a methyl group absorption. This band in isobutane is double—one component at 1375 cm.$^{-1}$, the other at 1360 cm.$^{-1}$. This doubling is characteristic of a terminal isopropyl group.

However, spectral differences and not similarities are of prime importance in analytical work. It is immediately apparent that *n*-butane has a strong band at 975 cm.$^{-1}$, a region in which there is negligible absorption in isobutane. The reverse situation is true at 1180 cm.$^{-1}$ where the iso form has a band and the normal has not. Both these bands are seen to appear in the mixture. Therefore, an analysis for one component in the presence of the other is a simple

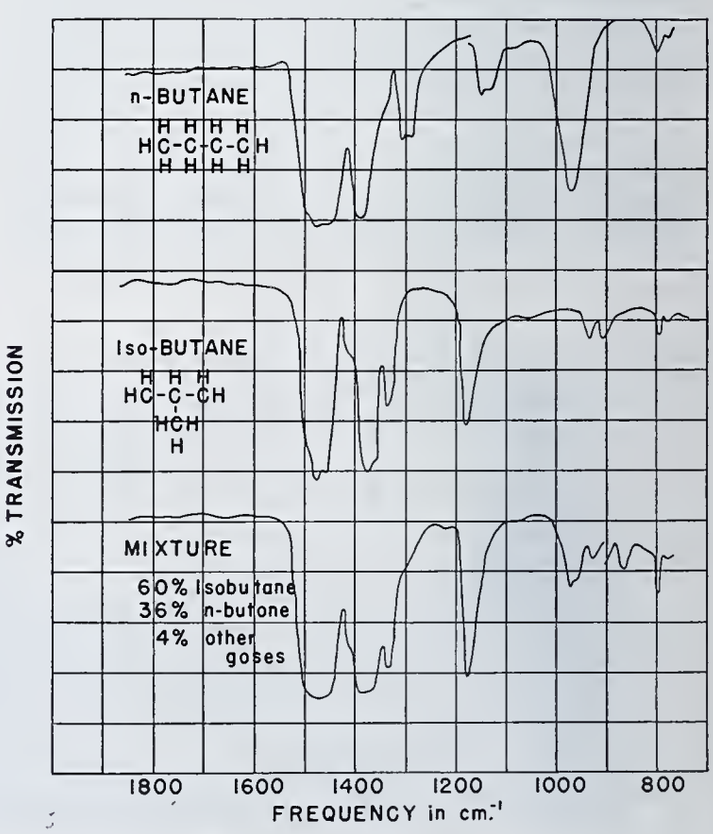


FIGURE 4. INFRARED SPECTRA OF PURE AND MIXED C_4 SATURATED HYDROCARBONS

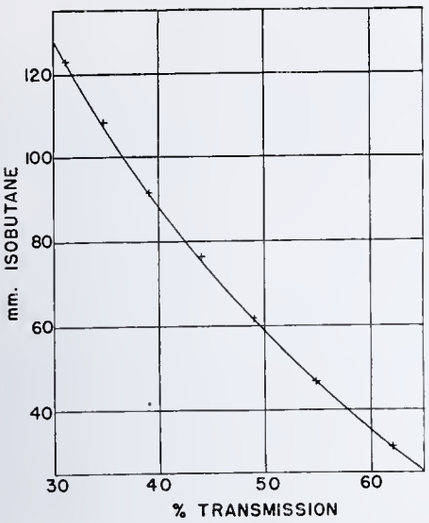


FIGURE 5. WORKING CALIBRATION CURVE AT 1180 CM.$^{-1}$

Per cent transmission of various pressures of isobutane in a mixture of approximately 150 mm. of butane + isobutane. Scattering of points is less than 1 mm. in 150, or an accuracy of better than ±1 % of total composition.

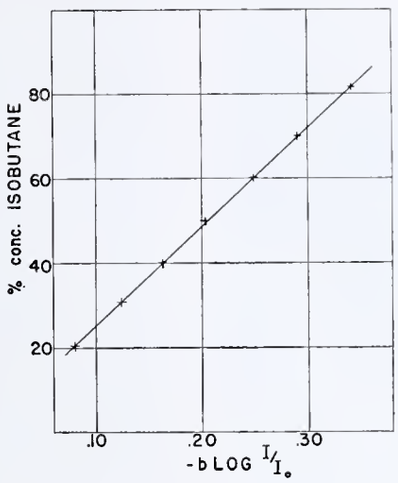


FIGURE 6. GRAPH OF FIGURE 5 REDUCED TO A STRAIGHT-LINE PLOT OF LOG PER CENT TRANSMISSION *vs.* ISOBUTANE CONCENTRATION

Values of Figure 6 have been corrected for scattered light and variation in total pressure. Constant b includes cell length, total pressure, and absorption coefficient K.

TABLE I. ISOBUTANE CONTENT

Chemical	Spectroscopic
%	%
38.3	38.3 (standard)
20.8	21.3
0.0	0.26
43.9	42.7
48.8	48.7

matter according to the technique outlined above. The working calibration sheet shown in Figure 5 was obtained by setting the instrument for the isobutane band at 1180 cm.$^{-1}$, filling an absorption cell with mercury manometric measures of isobutane, making up the mixtures with n-butane to about 150-ml. total pressure, and determining the per cent transmission of the prepared samples. A smooth curve drawn through these points shows a scattering of less than ±1 mm. of isobutane or an accuracy of better than ±1 per cent of the total mixture, in spite of the rough method of filling the cell. The time to obtain this curve was 3 hours. After this working sheet is once obtained, the time to analyze any unknown after the cell is filled would be no more than 5 minutes. If the n-butane content is also desired, another 5 minutes is required for a measurement at 975 cm.$^{-1}$. Incidentally, it is not essential for an analysis that one component have a negligible absorption at the frequency for which the second absorbs strongly. Actually, the accuracy of analysis at any frequency is a linear function of the difference in absorption coefficients (k) of the two components at this frequency. It is in order to make this difference as large as possible that analyses are made at that frequency for which one component has a strong absorption while that of the other is negligible.

In order to illustrate the method of working with a log per cent transmission plot, the values of Figure 5 were corrected by a constant factor for scattered light, cell window absorption, and variation in total pressure; and the straight-line graph of Figure 6 was plotted. This method of treating the data is theoretically much more rigorous. The resultant accuracy (or point scattering) is the same in Figure 6 as in Figure 5, although a greater time is required to prepare the calibration sheet. However, the method of log I/I_0 plotting is useful for extrapolation to a range of concentration for which calibration standards might be difficult to procure.

With respect to this particular mixture, the authors measured some samples for their isobutane content, in order to compare results with those obtained by fractional distillation. Here one sample was taken as a standard and the method of Figure 6 was used for measuring the others. Table I gives the results for each method of analysis. This comparison offers a further proof of the accuracy of spectroscopic methods.

Figure 7 shows the spectra of the unsaturated C_4 hydrocarbons. It was pointed out above that a double bond would be a much stronger "spring" than a single bond. This stronger spring is apparent in butene-1, butene-2, and isobutylene, where a C=C vibration occurs at 1650 cm.$^{-1}$. In butadiene, however, the presence of two strong springs connected by a weaker one causes two strong bands, one at 1825 cm.$^{-1}$, the other at 1600 cm.$^{-1}$. The butene-1 was rated at 95 per cent purity. Because of the presence of the weak bands at 1825 cm.$^{-1}$ and 1600 cm.$^{-1}$, it is likely that butadiene is a major impurity in the butene-1.

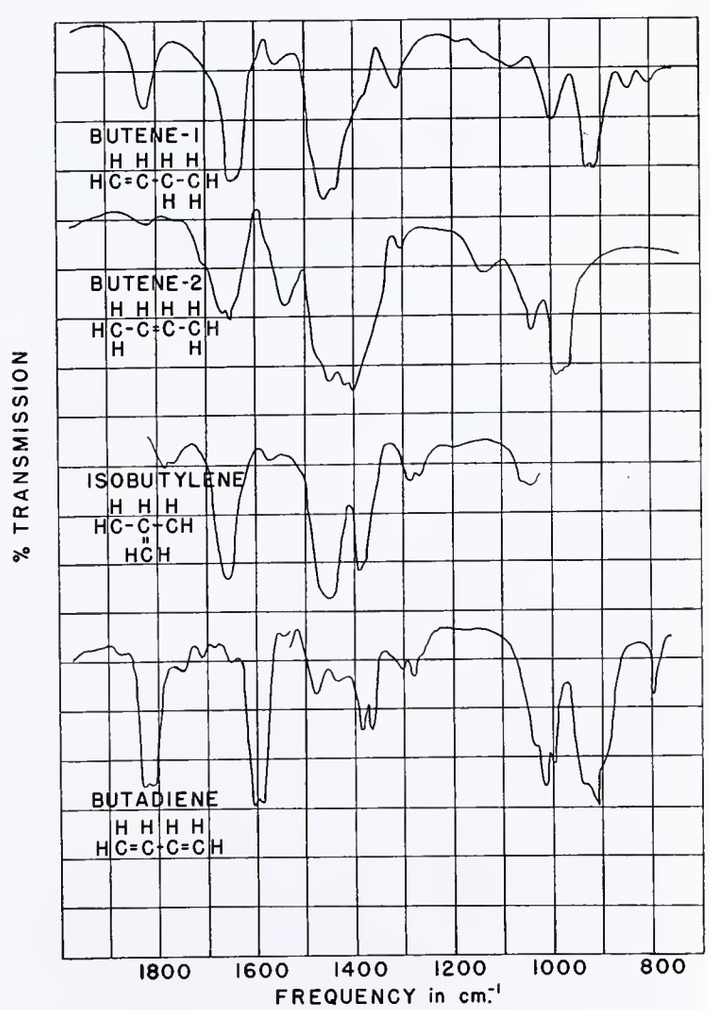


FIGURE 7. INFRARED SPECTRA OF C_4 UNSATURATED HYDROCARBONS

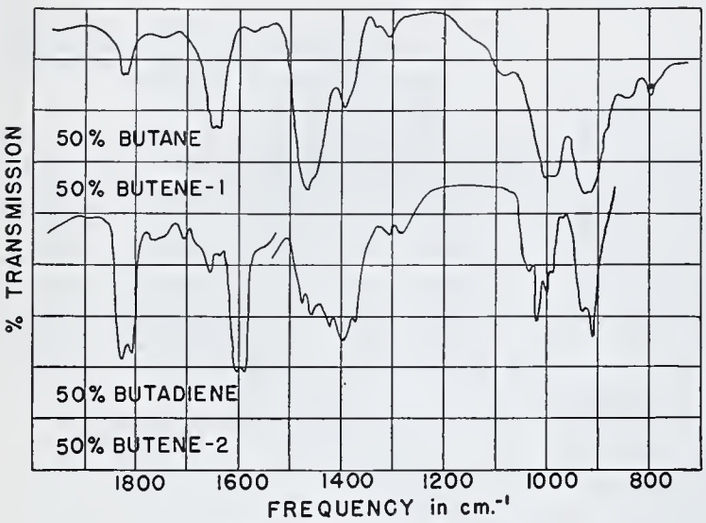


FIGURE 8. INFRARED SPECTRA OF MIXED UNSATURATED C_4 HYDROCARBONS

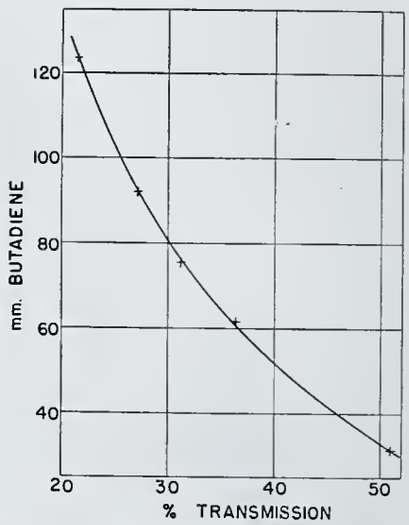


FIGURE 10. WORKING CALIBRATION CURVE AT 1825 CM.$^{-1}$

For partial pressure of butadiene in approximately 150 mm. of butadiene + butene-2

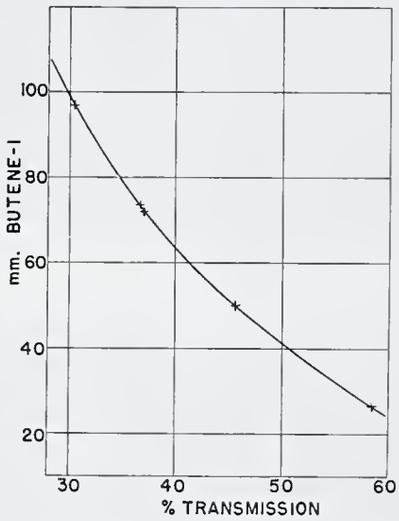


FIGURE 9. WORKING CALIBRATION CURVE AT 1650 CM.$^{-1}$

For partial pressure of butene-1 in approximately 120 mm. of butene-1 + *n*-butane

These higher frequency absorptions can be used in analyzing unsaturated hydrocarbons for the presence of saturated components, or monoolefins for the presence of conjugated olefins. The spectra of such mixtures are shown in Figure 8. Figures 9 and 10 are working sheets prepared for a series of mixtures of butene-1 in *n*-butane, and butadiene in butene-2. Again, the scattering of these points shows that the accuracy of analysis for one component in this range is better than ±1 per cent of the total mixture.

The authors have tried to illustrate the applicability of infrared spectroscopic analysis to the essential steps in the preparation of butadiene for rubber synthesis. There are two further considerations which should be discussed.

It is apparent from the examples chosen that the contents of the binary mixtures shown above can be analyzed quickly and accurately. The question may arise as to the possibility of a complete analysis of a mixture of all C_4 hydrocarbons which may result from one process. The success of such an analysis cannot be predicted from an examination of the spectra shown. It may not be possible to find a band for each component which is unique to that component. However, there is the possibility of finding a band which is common to two components and a band unique to one of these two. The second component can then be found by a difference method. An extension of this method may lead to the necessity of allowing for three or four components at one frequency. Such a situation may tend to lower the over-all accuracy of the analysis to a slight extent. However, this method can be used without appreciable loss of time. In

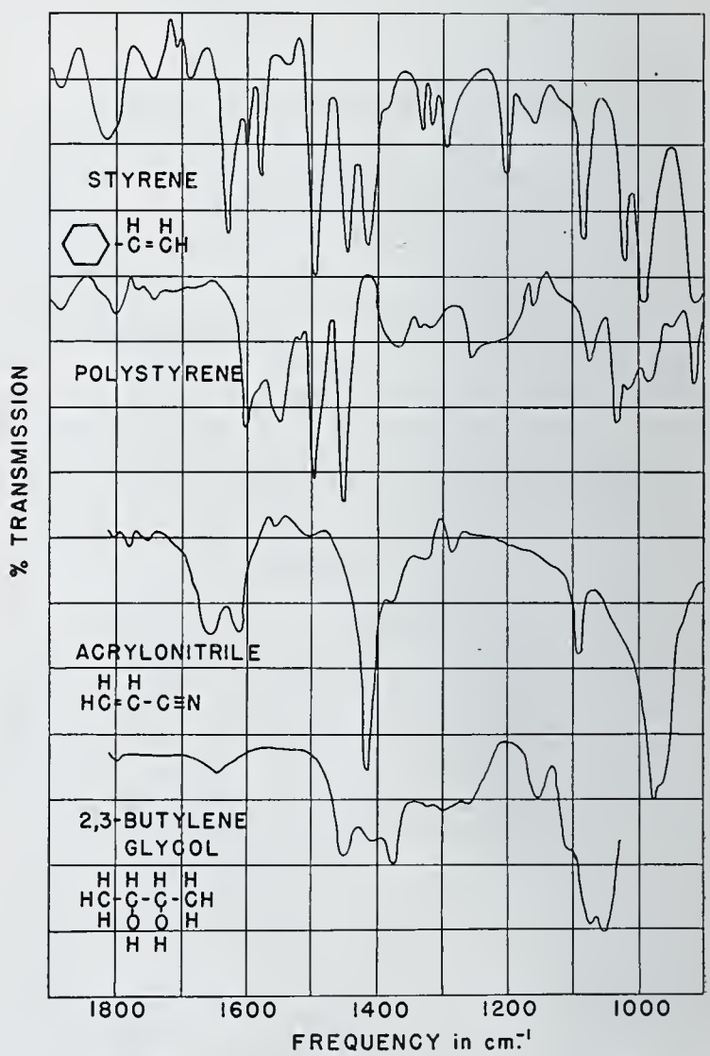


FIGURE 11. INFRARED SPECTRA OF SOME INTERMEDIATES IN PRODUCTION OF SYNTHETIC RUBBER

Samples studied as liquid or solid films

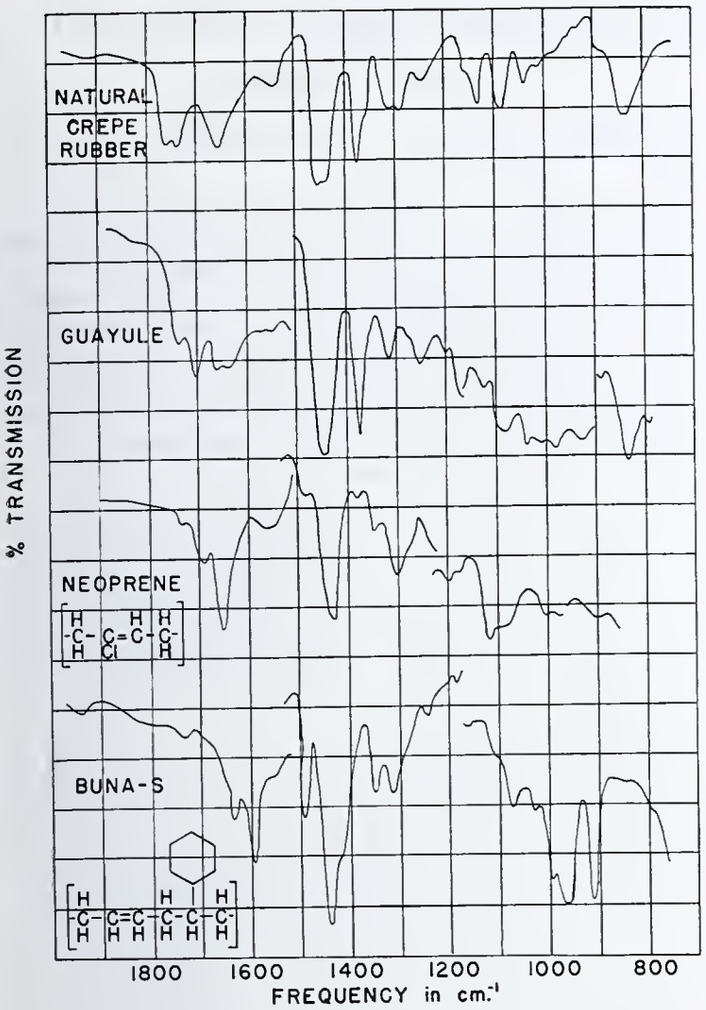


Figure 12. Infrared Spectra of Natural and Synthetic Rubbers

fact, a routine analysis performed in this laboratory measures the presence of six impurities in a mixture with an accuracy of ±1 per cent of the total mixture.

The second point concerns the fact that the working sheets shown measure the presence of one component between 20 and 80 per cent concentration. It can be seen from the steepening slope above 80 per cent concentration or from a consideration of the exponential nature of Beer's law, that the accuracy of direct analysis for one component falls off as the concentration of that component approaches 100 per cent. In fact, for such high concentrations it is more accurate to analyze for the lesser components and obtain the major component by difference. Below 20 per cent, the accuracy of analysis for a component increases markedly. Consider an analysis at 1180 cm.$^{-1}$ for a 1 per cent concentration of isobutane in *n*-butane. Since the absorption of the *n*-butane is practically negligible, the absorption cell can be filled to atmospheric pressure. Isobutane will be present to 7.6-mm. pressure and can be measured with an accuracy better than ±1 mm. Hence the accuracy for this concentration of isobutane is better than 1 part in 760 or approximately ±0.1 per cent of the total mixture.

The infrared spectra of a few other intermediates in the production of synthetic rubber are shown in Figure 11. The spectrum of polystyrene has been included to illustrate the spectral changes which occur upon polymerization. The monomer styrene bands at 1625 cm.$^{-1}$, the C=C vibration, and at 1410 cm.$^{-1}$, the bending vibration of the vinyl C=H group, have completely disappeared in the polymer. These unique bands are of great value in measuring the amount of monomer present in a monomer-polymer mixture. By extracting time interval samples from a polymerization reaction, the rate of reaction under various experimental conditions can be measured quickly and accurately. The method suggests itself as a possibility for measuring reaction rates in rubber synthesis. A characteristic band of acrylonitrile is the C≡N absorption at 2240 cm.$^{-1}$ (this region is not shown in Figure 12). Since this chemical group is not affected in the synthesis of Perbunan and Hycar, it is found to reappear in the spectra of these rubbers. Again a unique band is offered for quantitative work. The spectrum of 2,3-butylene glycol shows little absorption in the unsaturated region from 1900 cm.$^{-1}$ to 1600 cm.$^{-1}$. An analysis for unsaturated materials in this compound is therefore readily possible.

In order to show that infrared spectroscopy can also be used to derive information concerning some natural and synthetic rubbers themselves, several spectra are shown in Figures 12 and 13. (The formulas for the unit structure of these compounds were taken from Powers, *6*.)

These spectra exhibit marked differences, so that an unknown rubber can be identified by comparison of its spectrum with that of a known sample. Moreover, the spectra offer information concerning the structure of the molecules themselves.

These samples of rubber were the purest ones available to the authors at the time. Their history is not well known, and some oxidation may have occurred which would lead to the presence of spurious bands in the spectrum. The general spectrum, however, must be close to that of the

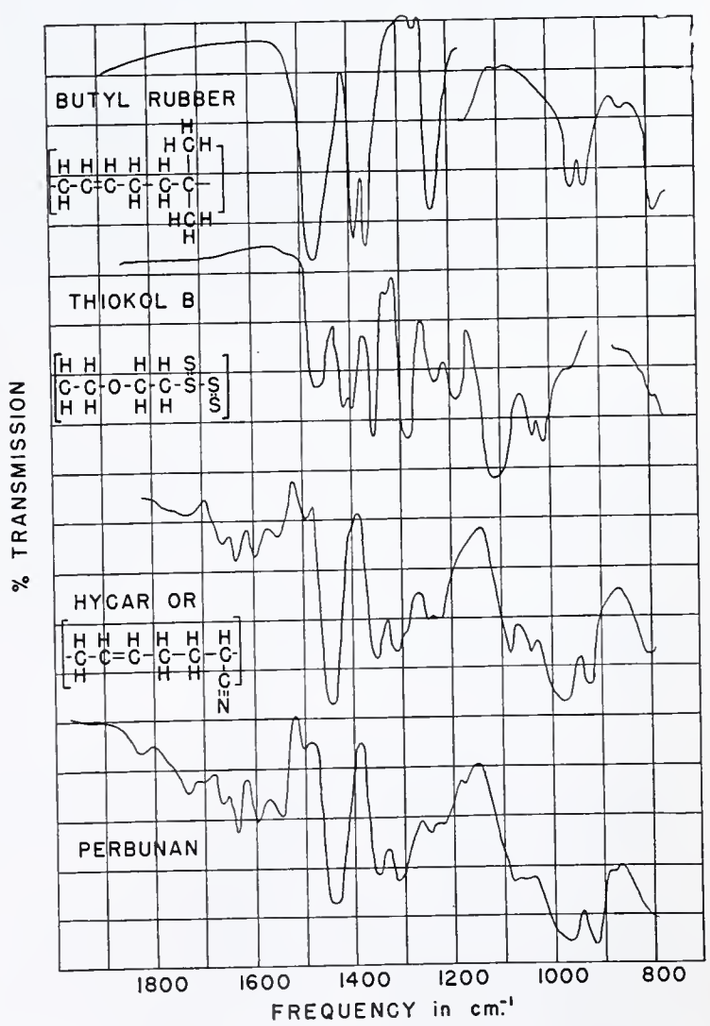


Figure 13. Infrared Spectra of Synthetic Rubbers

pure substance. The method of preparing samples for study was to form a "gluey" state of the rubber by mixture with a suitable material as carbon tetrachloride or acetylene tetrachloride. A film of this glue was spread on a rock salt plate and the solvent was evaporated.

Strong bands are observed in the spectrum of pure natural crepe rubber at 1750 cm.$^{-1}$, 1610 cm.$^{-1}$, and 1375 cm.$^{-1}$. It was known that this sample was 6 years old. This fact is evident in the spectrum, for the C=O band at 1750 cm.$^{-1}$ shows that considerable oxidation has taken place. The strength of this band could be used to make a quantitative analysis of the amount of oxidation in a sample for correlation with elasticity or abrasion tests. The 1610 cm.$^{-1}$ band is probably caused by a C=C bond while the 1375 cm.$^{-1}$ absorption points to the presence of methyl linkages. The small shoulder at 1360 cm.$^{-1}$ suggests that some of these may be terminal isopropyl groups. Somewhat similar bands are seen in the spectrum of guayule, although the greater number of absorptions in the region from 1600 cm.$^{-1}$ to 1800 cm.$^{-1}$ points to a greater complexity of unsaturated material in the guayule than in the crepe rubber.

The bands at 1650 cm.$^{-1}$ in Neoprene GN and at 1635 cm.$^{-1}$ in Buna S are caused by the presence of the C=C linkage. Neither compound has indication of methyl groups at 1375 cm.$^{-1}$. Butyl rubber and Thiokol B show no absorption in the 1650 cm.$^{-1}$ region, suggesting that there is far less unsaturation present in these samples than in the previous two. Butyl rubber has a peculiar double band at 1375 cm.$^{-1}$. This band splitting is different from that exhibited by an isopropyl group and may be characteristic of a tertiary butyl group. The bands at 1595 and 1495 cm.$^{-1}$ observed in Buna S are characteristic of an aromatic ring. The presence of these bands in an unknown rubber sample would immediately label it as a styrene-type rubber. The spectra of Perbunan and Hycar OR are seen to be very similar in frequency absorption, although the relative intensities of the bands are different. This similarity is not surprising, since the Perbunan is approximately 25 per cent acrylonitrile and 75 per cent butadiene, while the ratio is 40 per cent to 60 per cent in Hycar. The strong 1825 cm.$^{-1}$ band of butadiene has disappeared, since the conjugation has been broken in synthesis. As pointed out above, these two spectra show the C ≡ N band at 2240 cm.$^{-1}$, which provides an immediate tag for acrylonitrile rubbers.

Summary

Rapid quantitative analysis of compounds of prime interest in the production of synthetic rubber is possible using a relatively simple technique of spectroscopy. An apparatus suitable for such analyses is described, which does not require skilled technicians. It may be easily converted into an automatic control device for the control of production. The spectra of the natural and synthetic rubbers illustrated provide information concerning the structure of the rubber molecules and could be used to identify the type of rubber present in unknown samples.

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A Vacuum Take-Off Receiver for Solids

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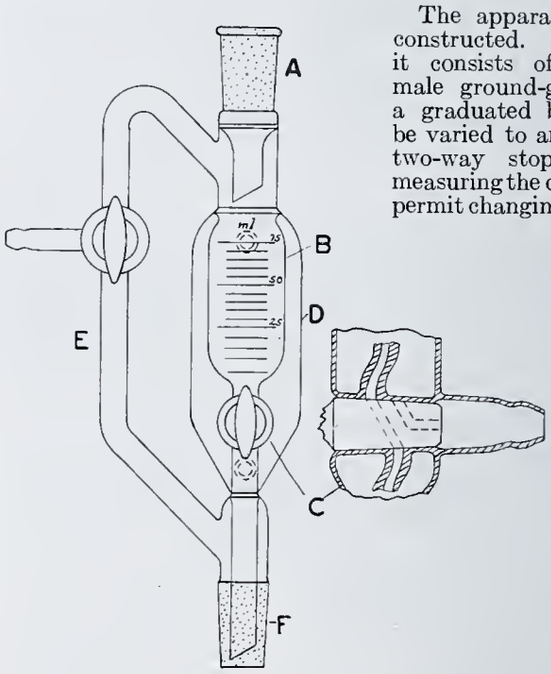
MANY satisfactory distillation receivers for glass laboratory stills are available for measuring and collecting distillates which are liquids at ordinary temperatures. Those designed, however, for distillates which are solids at ordinary temperatures usually leave much to be desired. After many trials, a receiver was designed which has given excellent results in the distillations of such materials as naphthalene, diphenyl, anthracene, and phenanthrene. An outstanding feature is a unique two-way stopcock arrangement.



The apparatus is sturdily constructed. As illustrated, it consists of a 24/40 female ground-glass joint, *A;* a graduated barrel, *B* (can be varied to any size), and a two-way stopcock, *C*, for measuring the distillate and to permit changing the collection flask; a jacket, *D*, enclosing both the barrel and the stopcock, to permit circulation of a suitable liquid at any desired temperature; an equalizing line with a two-way stopcock, *E;* and a 24/40 male joint, *F*, for connecting a collection flask.

Stopcock *C*, as shown in the diagram, has a tapered glass tail for a hose connection blown directly onto the end of the barrel casing. Stopcocks of this type usually have an extended barrel to which the hose is attached or have a small extension out of the top of the barrel casing. The first type is unsatisfactory, as turning the cock causes the hose to twist and often results in breaking the stopcock seal. The second type does not permit the stopcock to be jacketed. By the arrangement illustrated in *C*, the cock turns freely and there is no danger of loss of vacuum.

In actual distillation, when a fraction is to be taken, stopcock *E* is turned so that the lower portion of the equalizing line is isolated from the still; then stopcock *C* is turned to permit release of the vacuum in the collection flask; after a new receiver is put in place, the hose connected to *C* is attached to a vacuum line and the pressure is reduced; the stopcocks then are returned to their proper positions.

The apparatus is not limited to the collecting of solids or to distillations made at reduced pressures. It works equally as well at atmospheric pressure and when the proper cooling medium is circulated it is very satisfactory for use with low-boiling distillates.

Acknowledgment

The author wishes to acknowledge the helpful construction suggestions made by the Ace Glass Company, Vineland, N. J., which made the apparatus to the author's specifications.

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Lubricating Oil Detergency

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The significance of lubricating oil detergency is discussed with reference to various types of internal combustion engines. Four methods for evaluating this important property are described.

One is a chromatographic procedure for determining the depth of penetration through sand of lampblack suspended in the oil. Two other methods involve separation of asphaltenes from the oil, by centrifuging and by filtration through an asbestos mat, respectively. The fourth depends upon the relation between the degree of dispersion of oil-insoluble material and transmissivity to infrared radiation.

In the chromatographic test, lampblack was selected because of its similarity to the sooty fuel deposits in Diesel engines, and the laboratory results are in good agreement with experience on the Caterpillar Diesel. On the other hand, the procedures employing asphaltenes measure the peptization of oil oxidation products and correlate more nearly with ratings of the Lauson and certain other engine tests.

It is concluded that the laboratory tests are of interest in elucidating the nature of deposit formation in engines and, within limits, also provide a useful means for evaluating the detergent characteristics of lubricating oil additives. However, specific application of the tests requires consideration of the type of material deposited in a given engine as well as the operating temperature.

DETERGENCY is a necessary property of lubricating oils for use in internal combustion engines. Specifically, oils must have the capacity of carrying away soot and other combustion chamber detritus, as well as their own decomposition products, in order that engine deposits shall not accumulate and interfere with normal engine operation. This requirement creates a special problem in Diesels, which produce relatively large amounts of soot from incomplete combustion of the fuel, and it has been found necessary to incorporate detergent additives in oils for such service in order to avoid premature engine failure from ring sticking. High-output engines, which operate at high piston and cylinder wall temperatures, also create special detergency problems in regard to lacquering and ring sticking. For low-output engines, operating on clean burning fuels, the inherent detergency of plain mineral oil is usually sufficient to keep the engine clean.

While acceptable detergent additives have been on the market for a number of years, the development of newer and better additives has been hampered by the lack of simple laboratory methods of testing oils specifically for detergent properties. Engines have had to be used for this testing, even in the preliminary stages of testing experimental additives, although their use has simultaneously involved other variables, the least desirable of which has been a wide variation in quantity of material to be dispersed in supposedly comparable tests. Other reasons for desiring small-scale laboratory tests for detergency are small sample requirements, speed of testing, reproducibility, and at least a partial separation of variables. This paper describes four methods that have been found useful for investigating detergency on an empirical basis in this laboratory and the field of usefulness of each.

Detergency is complex and a single test cannot be expected to provide an absolute evaluation that will fit the diversity of conditions that prevail in an engine. From a physicochemical point of view it is a generic term and includes a variety of actions, such as solubilization, emulsification, and base exchange (*2*). The experiments indicate, however, that, in an engine, lubricating oil detergency acts in general to prevent deposition on solid surfaces rather than to remove deposits already formed. The success of a test depends upon a judicious compromise of a number of effects and its usefulness upon an understanding of the limitations of each method. The greatest difference found in the detergency requirements of lubricating oils lies in the fact that the materials to be dispersed vary from soot (derived from fuel) to asphaltenes (derived from oxidized lubricants).

The difference in action of low viscosity index oils (in the range 30 to 60) and high viscosity index oils may be mentioned as an example of the complex nature of lubricating oil detergency. There is generally no difficulty in securing representative samples during the course of oxidation of high V. I. oils in the Indiana oxidation test and there is considerable assurance that each such sample will contain a proportionate part of the oil oxidation products. In contrast, low V. I. oils do not so readily maintain oxidation products in suspension, and serious errors in analysis can result when an attempt is made to withdraw representative samples; a large part of the oxidation products separate out and adhere firmly to the glass container. This same difference in detergent action is reflected in Lauson engine tests for lacquering tendencies. Low V. I. oils generally begin to deposit lacquer on the piston skirt, etc., from the start of the test, while high V. I. oils have an induction period during which concentration of oxidation products builds up in the oil while little or no lacquering takes place and then, depending on the engine conditions, oil-insolubles may even remain at a relatively constant value after the deposition of lacquer is well established. From this picture it would appear that high V. I. oils have a greater detergency than low V. I. oils; however, it is for precisely the reverse reason that undoped low V. I. oils are considered best for lubricating Diesel engines. This anomaly may eventually be explained on the basis of factors other than detergency.

As an example of the complexity of detergent action in another field, McBain and others show that the detergent action of potassium myristate is increased 20 times by a 50 per cent excess of potassium hydroxide and 4 times by a 10 per cent excess of myristic acid. Wide variations are also shown for change in the concentration of the neutral soap, the temperature, and even the method of making up the solutions (*3*). Similar effects probably exist in lubricating oil detergency.

In 1938, Bray, Moore, and Merrill (*1*) described a simple method for evaluating lubricating oil detergency, in which specimens of white cloth were soiled in a standard dispersion of lampblack in the lubricant under test. The soiled cloth was washed in an equal-volume mixture of oil and naphtha (Stoddard solvent) and then in naphtha alone until the washings were clear. The efficiency of the detergent agent was denoted by the grayness of the washed cloth in comparison with a blank test in which no additive was present. No study was made of this test

Table I. Effect of Heat on Oxidized Oil Solubility

(Centrifugal detergency test)

Procedure	Treatment before Centrifuging: Mixing, Hours	Mixing, ° C.	Storage[a], Hours	Storage, ° C.	Oil-Insolubles, in Reference Oxidized Oil[b], % by weight
A	2	30	..	..	0.81, 0.82, 0.83
B	10 min.	30	1	110	0.66
C	1	110	1	110	0.65, 0.66
D	1	150	2	30	0.62
E	1	150	1	150	0.58, 0.62
F	1	250	1	250	0.86, 0.82

[a] In centrifuge tube just prior to centrifuging, no stirring.
[b] SAE 30 Western oil, oxidized in Indiana oxidation apparatus and composited for these experiments.

in the present investigation, because the dilution with naphtha introduced an undesirable factor of uncertainty.

A simple setting test for detergency was examined in some detail. In this test, oils were shaken up with 0.2 per cent by weight of lampblack and allowed to stand at room temperature, and observations were made of the settling times to give a clear meniscus, a cloudy oil, and a clear oil. The test was applied to thirty oils of comparable viscosity, but of different base stocks and with different additives. There was no correlation of the rating given the oils in this test and the rating obtained with general engine experience. In fact, some very effective detergents were rated poor and a few plain oils without detergent additives were rated excellent.

A static peptization test was also tried without success. In this test, about 0.3 gram of powdered asphaltenes was placed in a small vial and 10 ml. of oil to be tested were gently added. Once each day the vial was gently inverted and inspected to see if any detergent action had taken place. There was no other stirring in the test. A series of oils was tested simultaneously on one rack and, while there was a difference in detergent action, the relative rating had no apparent significance.

The four tests discussed below were found to give useful information.

Centrifugal Detergency Test

The centrifugal detergency test is based upon the use of a high-speed centrifuge to determine the weight percentages of insolubles in oxidized oils without dilution. In order to test for detergency, a reference oxidized oil is run with and without the additive under test. The reduction, if any, of oil-insoluble material that results from the additive is taken as a measure of its detergency value. Since different oxidized oils are used from time to time, the results have relative significance only. The merit of this method of testing for detergency is that it measures one specific aspect only, the ability of the additive to increase the degree of dispersion of the oil-insoluble material beyond an arbitrary but presumably reproducible limit. Only indirectly does it measure the ability of an additive to displace oil-insolubles on a hot metal surface or to clean them off after they have been deposited.

Oil-insolubles are determined without dilution as follows:

The oil sample is thoroughly shaken, and 20 to 25 grams are weighed into a clean, dry centrifuge tube to 0.01 gram and centrifuged for 45 minutes in a de Laval 100-ml. precision centrifuge (10,000 × gravity). This time is satisfactory for almost all oxidized SAE 30 and lighter-viscosity oils; longer times are required for the more viscous oils and for oils that do not settle readily. The centrifuged oil is poured out of the tube in such a manner as to disturb the precipitate as little as possible. The tube is clamped in an inverted position and heated gently with a Bunsen burner, and as much oil as possible is drained from the tube. After cooling to room temperature, 50 ml. of isopentane are added and the precipitate is broken up with a rod or wire and thoroughly dispersed in the wash liquid. The tube is centrifuged for 15 minutes to repack the precipitate. All but about 0.5 ml. of the isopentane is removed with a pipet and the washing procedure is repeated with a second 50-ml. portion of isopentane. The washed precipitate is allowed to stand at room temperature until all visible isopentane has evaporated. The precipitate is then dried at 100° C. for 1 hour, brought to room temperature, and weighed in the centrifuge tube to 0.1 mg. The results are reported as percentage of oil-insolubles on the basis of the oil charged into the centrifuge tube.

The results of heating an oxidized oil to different temperatures before centrifuging for oil-insolubles are given in Table I.

Mild heating to 110° or 150° C. increases the degree of dispersion of oil-insolubles; but higher temperatures, in the range of 250° C., apparently accelerate coagulation, and the quantity of centrifugally separable oil-insolubles is increased. Stirring or storage for 1 hour at room temperature does not alter the increased dispersion brought about by mild heating.

Table II shows the effect of diluting an oxidized oil with various fresh oils on the dispersion of oil-insolubles. Dilution of the oxidized Western oil with an equal quantity of fresh oil of the same kind does not alter the degree of dispersion of the oil-insoluble material at any temperature. In other words, fresh and oxidized Western oils are compatible as regards the precipitation of oil-insoluble materials. In contrast, when fresh Mid-Continent and Pennsylvania oils are added to the oxidized Western oil, the dispersion of the oxidation products is decreased and precipitation of these materials could occur. However, this ill effect is reduced when the diluted oils are heated to 100° C. or above. A similar effect is indicated for the more viscous Mid-Continent oil, but the reproducibility of the test is poor and the result is somewhat uncertain.

As shown in Table III, detergent additives increase the dispersion of oil-insolubles. Except in the case of the low-tem-

Table II. Effect of Dilution with Fresh Oil on Solubility

(Centrifugal detergency test)

Procedure	Treatment before Centrifuging: Mixing, Hours	Mixing, ° C.	Storage[a], Hour	Storage, ° C.	Oil Insolubles[b]: Reference oxidized oil[b]	Reference Oxidized Oil Diluted with Equal Volume of Fresh[c]: Western 30	Mid-Continent 30	Penn 30	Mid-Continent 60
					Per cent by weight				
A	2	30	.	...	0.81, 0.82, 0.83	0.86, 0.91	0.94, 1.01	1.01, 1.02	
C	1	110	1	110	0.65, 0.66	0.74, 0.78	0.90, 0.93	0.63, 0.89, 0.92	0.77, 0.89
E	1	150	1	150	0.58, 0.62	0.62, 0.63	0.80	0.76, 0.74	0.82

[a] In centrifuge tube just prior to centrifuging, no strirring.
[b] All oil-insolubles given on basis of undiluted oxidized oil.
[c] SAE Western oil, oxidized in Indiana oxidation apparatus and composited for these experiments.

Table III. Effect of Additives on Oil Solubility

(Centrifugal detergency test)

Procedure	Treatment before Centrifuging: Mixing, Hours	Mixing, ° C.	Storage[a], Hour	Storage, ° C.	Oil-Insolubles for Reference Oxidized Oil[b] Containing: Nothing	1% low temperature detergent	1% reference detergent	1% high temperature detergent[c]	1% benzene
					Per cent by weight				
A	2	30	...	...	0.81, 0.82, 0.83	0.70	0.84, 0.85	0.86	..
C	1	110	1	110	0.65, 0.66	0.48	0.50, 0.52	0.56	0.66
E	1	150	1	150	0.58, 0.62	0.46, 0.46	0.45, 0.47	0.49	0.64
F	1	250	1	250	0.86, 0.82	1.14, 1.03	0.44, 0.35	0.49	..
G	20 min.	300	...	...	.....	1.16	0.58	0.47	..

[a] In centrifuge tube just prior to centrifuging, no stirring.
[b] SAE 30 Western oil, oxidized in Indiana oxidation apparatus and composited for these experiments.
[c] Added in benzene solution.

perature detergent, mild heating is required to achieve the best results. Some detergents have a distinct temperature limitation and their use may actually increase the quantity of oil-insolubles that separate if they are heated to too high a temperature. Both the reference detergent, which has been found satisfactory for commercial use, and the high-temperature detergent show a useful effect even when heated to 300° C. for a short time. The effect of benzene is included in the table because some additives are blended into lubricating oil in the laboratory in this solvent. As can be seen, its effect is negligible.

The three detergent additives have been tested in several Diesel engines and an effectiveness corresponding to the indications of the solubility data in the table has been noted at high engine temperatures. For example, the ring sticking time in the Fairbanks-Morse Diesel engine operated under severe conditions was increased two times with the reference detergent and ten times with the high-temperature detergent as compared with the base oil; the low-temperature detergent was not tried in this engine.

Table IV. Infrared Detergency Test

	Fresh Oil	Oxidized Oil A	B	C
Oil-insolubles, % by weight, centrifuge				
Plain	...	.....	0.41	0.79
With 1% high-temperature detergent	...	.....	0.19	0.60
Infrared transmission, % 9700 Å.[a]				
Plain	100	9.4, 7.8	14.5	5.0
With 1% high-temperature detergent	97	31.0, 41.0	29.7	5.6

[a] Not centrifuged.

Infrared Detergency Test

Since the degree of dispersion of a solid suspended in a liquid is associated with the ability to transmit light, the light-absorption curves of a few fresh and oxidized oils were examined in a Coleman electric photometer. They all showed a pronounced transmission band at 9700 Å. and this wave length was arbitrarily selected for examining the effect of additives on the dispersion of oil-insolubles.

In Table IV are given results obtained with one additive on three laboratory oxidized oils, chosen at random, and the original fresh oil. In all cases, the infrared light transmission of the oxidized oil was increased by simply adding a detergent to the oil sample after oxidation, whereas the transmission of the fresh oil was slightly reduced. The effect of this same additive in the centrifugal detergency test on two of these oils is included for comparison. It is concluded that transmission at this wave length is associated with an increased degree of dispersion of the insolubles involved and it would appear that the ultimate particle size achieved by the dispersive action of the detergent is very small. This follows from the fact that transmission of light is a function of scattering as well as true absorption. Assuming that absorption is negligible because of the selection of the wave length and that the scattering of light depends on particle size only, it follows that the particle size must have been reduced to a value commensurate with the wave length of light used in this test. A few other additives were examined by the infrared detergency test and all showed similar increases in light transmission when added to oxidized oil.

While the infrared detergency test has some interesting possibilities and should prove useful for securing information that cannot be obtained in other ways, it was abandoned in favor of the centrifugal detergency test because the results of the latter could be interpreted with less uncertainty. Both tests presumably measure similar actions of detergents.

Wood River Detergency Test

A detergency test was investigated and developed by F. L. Johnston, J. B. Harkness, and G. A. Siegelman of the Wood River Research Laboratories of the Shell Oil Company, Incorporated. In it, the percentage of added asphaltenes that a given oil (and additive) will carry through an asbestos filter is taken as a measure of the detergency of the oil.

Experimental Procedure. To a 20-gram sample of the oil in a test tube (15 × 150 mm.) are added 2 ml. of benzene solution containing 20 mg. of asphaltenes. The sample is shaken vigorously, placed in an oil bath, and aged at 150° C. for 16 hours. The sample is removed, shaken again, allowed to cool to room temperature, and filtered through a dried and tared Gooch crucible with an asbestos pad. The precipitate is then washed exhaustively with 60–80 naphtha to remove the oil, and the crucible is dried for 1 hour at 110° C. (230° F.), allowed to come to room temperature, and again weighed. The percentage peptization is computed from the difference between the observed gain in weight and that which would occur if there were no peptization, using the following equation:

$$\% \text{ detergency} = 100\ 1 - \left(\frac{\text{weight of asphaltenes in crucible}}{\text{weight of asphaltenes added}}\right)$$

The asphaltenes are prepared by diluting an oxidized oil with naphtha, filtering and washing the precipitate, and removing the asphaltene fraction by benzene extraction. For special investigations it may be desirable to vary the procedure and add solid asphaltenes free of a solvent. Great care must be taken in removing the benzene because large variations in asphaltene solubility can result from even mild overheating. A practical procedure is to weigh out about 20 mg. of dry asphaltenes to 0.1 mg. and add 1000 times this weight of the fresh oil to be tested.

Since the quality of asphaltenes will vary greatly from one preparation to another, the results can have relative significance only. If metal salts are present in the asphaltene preparation, certain anomalies may be encountered in testing some additives and, for that reason, oil samples oxidized in the laboratory in the absence of metals are recommended as a source of asphaltenes for this work.

Table V. Effect of Naphthenate Additions

(Wood river detergency test)

Oil	Detergency Rating, %
Plain mineral oil, Mid-Continent SAE 10	22
Oil + 0.5% by weight sodium naphthenate	49
Oil + 0.5% by weight barium naphthenate	78

Table VI. Comparison of Laboratory Rating and Engine Lacquer Rating

(Wood River detergency test)

Detergent	Concentration *% by weight*	Detergency %	Lacquer Reduction in Lauson Engine %
A	1.0	64	89
B	2.0	58	50
C	2.0	40	38
D	1.4	14	13

Additional agitation during the aging period has been found to produce no significant effect. The substitution of a nitrogen atmosphere over the sample during the aging likewise has no effect, although a loss of detergency, probably through the destruction of the additive as well as an increase in asphaltenes, may result if oxygen is bubbled through the sample during the 16-hour aging period. In general, the effectiveness of a detergent increases up to 0.5 or 1 per cent concentration, after which the detergent action is constant for a given additive and is then mainly dependent upon the solubility distribution of the asphaltenes used.

In Table V is shown the increase in detergency secured by the addition of a sodium naphthenate and a barium naphthenate to a SAE 10 oil. Table VI shows the improvement obtained in the detergency rating in this test by the addition of several detergents to one base oil and the corresponding improvement in the Lauson engine lacquer rating.

Chromatographic Detergency Test

The chromatographic detergency test was developed here following a verbal report that General Motors were using a filtration test to measure the ability of an oil to hold soot and other engine deposits in suspension. In their test, a dirty used oil that remained dirty after filtering through a Gooch crucible filled with asbestos was given a "one star" rating. In the same way by passing the dirty filtrates through additional clean crucibles, the oil was given up to a maximum of a "three star" rating.

In order to provide a more discriminating test for development research and to reduce the maximum of three filtrations to one, a glass tube was substituted for the Gooch crucible in the General Motors test. A number of filtering media were studied, using lampblack suspensions in oils known to have widely different detergent activities. Lampblack was chosen for the early experiments because of its similarity to the soot of Diesel engines and was retained because it gave useful correlations.

The experiments showed that the depth of penetration of the lampblack could be readily observed and that the penetration was greatest for those oils rated most detergent by general Diesel engine experience. For a given oil, the depth of penetration varied greatly from one filtering medium to another but appeared to be largely dependent upon the coarseness of the packing. The relative ratings of the representative oils tested were roughly the same in different filtering media.

The actual limit of lampblack penetration in a homogeneous filter bed is not always clear, especially in the longer penetrations. It does not terminate abruptly, but tapers off in a poorly defined zone of diminishing intensity.

Table VII. Relative Rating of Mineral Oils

(Chromatographic detergency test)

Oil	Number of Darkened Rings SAE 10	SAE 30	SAE 70
Mid-Continent	1	3, 4	6, 6
Western	2	3, 2, 3	2, 3

In order to give a clear index of penetration, disks of filter paper are placed in the filtering tube at regular intervals. The edges of these papers are usually either distinctly blackened or not visibly altered by the lampblack, and the number of filter paper disks that have been darkened at the end of a test is taken as a measure of detergency. When tests are to be run at 150° C. or higher, layers of calcium carbonate powder are substituted for the paper, since paper chars when heated. The calcium carbonate-layered tubes are more difficult to prepare and unless the calcium carbonate is very carefully washed free of fines, undiluted oils cannot be filtered at room temperature.

The chromatographic test for detergency is carried out in Pyrex tubes, 38 cm. long by 7 to 8 mm. in inside diameter and constricted slightly at the bottom end. These are fitted with a cotton plug, on which are packed 10 or 12 alternate layers of sand and filter paper disks. Common sea sand is washed, ground, and separated to a particle size range of 15 to 150-micron average diameter; 0.4 ml. is used in each layer. Whatman's No. 4 filter paper is cut with a cork borer into disks approximately 8.4 mm. in diameter. The packed tubes are adjusted for moisture content for 2 hours just prior to making a test, by drawing through them air that has first been passed through 22 per cent potassium hydroxide solution and an adequate spray trap. To 10 grams of oil to be tested are added 20 mg. of Monsanto Germantown Bear Brand lampblack. The mixture is stirred vigorously for 5 minutes at room temperature and 1 ml. is measured and transferred into the filtering tube by means of a hypodermic syringe. The connection between the potassium hydroxide wash bottle and filtering tube is broken only long enough to add the sample to the latter. A vacuum of 60 to 67.5 cm. (24 to 27 inches) is maintained in the filtering tube for the duration of the test, 16 to 20 hours. The number of darkened filter paper edges is recorded at the end of the test.

Table VIII. Relative Rating of Detergent Additives

(Chromatographic detergency test)

Additive	Test Rating, No. of Darkened Rings	Caterpillar Engine Rating
None	2, 3	Poor
I	9, 11	O. K.
II	9	O. K.
III	7, 9	O. K.
IV	8, 8, 8	O. K.
V	7	O. K.
VI	6, 6	Insufficient detergency
VII	5, 4, 6	Insufficient detergency
VIII	4, 3	Poor
IX	6	Poor

Naphtha washing after oil filtration and before observing lampblack penetration is optional, but results between washed tubes cannot be compared with those from tubes that have not been washed with naphtha. Naphtha washing is usually desirable for the more viscous oils, SAE 40 and higher.

The calcium carbonate powder for high-temperature tests should be freed of fines by washing with naphtha and decanting repeatedly until all material that does not settle rapidly has been removed. Layered tubes of this sort are most readily filled by washing the solids into the tube in naphtha suspension and settling each layer with suction before adding the next. It is preferable to maintain a layer of naphtha above the packed solids in the tube until the packing is complete, after which the tubes are sucked dry and used without water conditioning. The tube with filter paper disks is packed dry with thorough tamping.

Of the variables that influence the penetration of carbon black in the filtering tube, water content has been found to be the most important for filtration at room temperature. While other factors, such as particle size of the filtering medium, relative quantities of materials used, degree of vacuum, etc., regulate the extent of penetration, the relative rating of a series of oils and additives can be altered by varying the moisture content of the tube packing. Water added to the suspension of lampblack in the oil also has a pronounced and sometimes erratic effect. Precautions for maintaining the moisture content of the filtering tubes constant are included in the described procedure and care should be exercised that water is not mixed in the oil. The observation that water affects the results of this test is important, because it is believed that traces of water have a similar important effect on lubricating oil detergency in engines.

Violent agitation, such as is produced by ultrasonic vibration of the lampblack suspension, increased the penetration of one sample from 7 to 11 rings. The maximum effect of an additive is generally reached at 0.5 to 1.0 per cent by weight concentration. With additive concentration at usual values, reduced penetrations may occur when the lampblack concentration is raised above 0.2 to 0.5 per cent by weight, although some additives can tolerate up to 2.0 per cent by weight lampblack with no pronounced reduction of effectiveness. Some additives begin to lose their effectiveness at 170° C. while others are fully effective, as measured by this test, up to 250° C.

Figure 1 illustrates the appearance of the filtration tubes in the chromatographic detergency test. This series of tubes was used to examine different batches of a particular additive. Batch 3 showed a definite superiority over the first two.

In Table VII are given the relative ratings of different grades of Western and Mid-Continent oils in this test. The higher rating given the more viscous Mid-Continent oils is not reflected in Diesel engine performance of these oils. The low rating given all three Western oils indicates that the relative

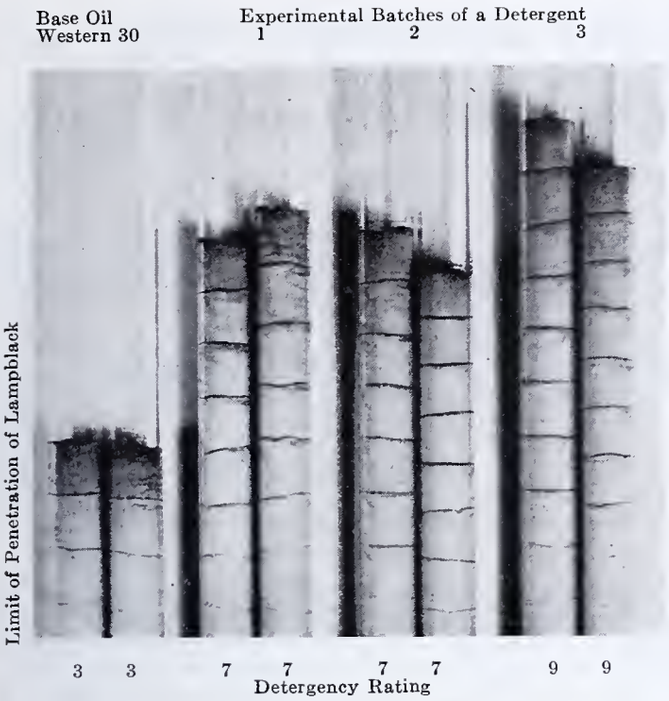

FIGURE 1. APPEARANCE OF CHROMATOGRAPHIC DETERGENCY TEST

Unequal height of sand layers in different tubes is caused by variations in tube diameters.

rating is not dependent upon viscosity differences as such. While the results of the chromatographic detergency test may be accepted as revealing a real difference in detergent properties of the oils examined, the results indicate that a high rating does not necessarily guarantee satisfactory Diesel engine performance. However, when a high rating is secured by an additive in a base oil of low rating, considerable reliance can be placed on improved engine performance as regards detergency, and the test is proposed only for this purpose. The data in Table VIII illustrate this use of the test and give an indication of the engine rating of the oils tested.

TABLE IX. COMPARISON OF CHROMATOGRAPHIC AND WOOD RIVER DETERGENCY TESTS

Additive	Chromatographic Rating, Darkened Rings		Wood River Rating	
	Eastern oil SAE 10	Western oil SAE 30	Eastern oil SAE 10	Western oil SAE 30
			%	%
None	1	1	51, 47	79, 83
1.4% detergent A	4, 2	6, 6, 5	−277	−100
1.0% low-temperature detergent	...	7, 6	...	−260, −290
2% detergent B	5, 6	7, 7	−14	2
1% detergent C	8, 7	7, 6, 8	81	75
10% voltol oil	...	6, 9	..	84
2.5% reference detergent	...	8, 8	..	76

Comparison of Wood River and Chromatographic Detergency Tests

The relative ratings of oils and additives are not the same in the chromatographic and Wood River detergency tests. Table IX gives a comparison of these two tests for several additives in two base oils. Only the last three additives are rated good in both tests, while the other three are rated fair to good in the chromatographic test and are given negative ratings in the Wood River test. The negative rating is taken to mean that additives as well as asphaltenes were removed on the asbestos filter in the latter test. Incidentally, additives that are effective as detergents in critical parts of an engine and yet aid in accumulating insolubles on a filter would be advantageous in engines, provided that the additive is not too quickly removed from the oil by the latter activity.

The lack of agreement in the ratings given by the two tests focuses attention on the necessity of considering two distinct types of detergency requirements. Indications are that the chromatographic detergency test correlates more nearly with the detergency requirements of the Caterpillar Diesel, while the Wood River test is more closely related to the lacquer rating of the Lauson engine. This difference appears more understandable when the predominant types of insolubles produced in the two engines are compared with the dispersed materials in the two tests.

Interchange of dispersed materials in the two tests provided no useful results. When lampblack is substituted for asphaltenes in the Wood River test, all detergency ratings are zero or negative. When asphaltenes are substituted for lampblack in the chromatographic test, the depth of penetration is poorly defined because the asphaltenes develop only mildly discolored rings at best. The complex character of the asphaltenes probably also contributes to the indifferent penetrations because of the range of specific penetrations involved.

While the laboratory detergency tests indicate a difference of detergent requirements for different classes of engine deposits, further studies must be made under conditions corresponding more closely to those parts of an engine that suffer most from a lack of detergency, in order that the questions raised may be answered more directly and reliably.

Conclusion

Four methods of evaluating the detergency of lubricating oils measure the ability of a lubricant to disperse or peptize oil-insoluble materials such as oxidized lubricants or soot. It is possible to use these tests profitably to predict the cleanliness-maintaining action of an oil in engines, providing consideration is made of the type of material most likely to be deposited as well as the temperature conditions under which the engine operates. In addition to their direct application to engines, which requires further correlation, they are useful tools in oil research and give quantitative data on many speculations made in the past on the detergent aspects of lubricating oils.

Acknowledgment

Grateful acknowledgment is made to H. C. Kennedy for his careful execution of the laboratory work described in this paper.

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CORRECTION. In the article entitled "Preparation of Diphenylthiocarbazide and Diphenylthiocarbazone" [IND. ENG. CHEM., ANAL. ED., 14, 953 (1942)], the last sentence in the first paragraph on page 954 should read as follows: "The yield is about 15 grams, 50 per cent of the theoretical based on the phenylhydrazine taken."

OLIVER GRUMMITT

Freezing Points, Densities, and Refractive Indexes of the System Glycerol–Ethylene Glycol–Water

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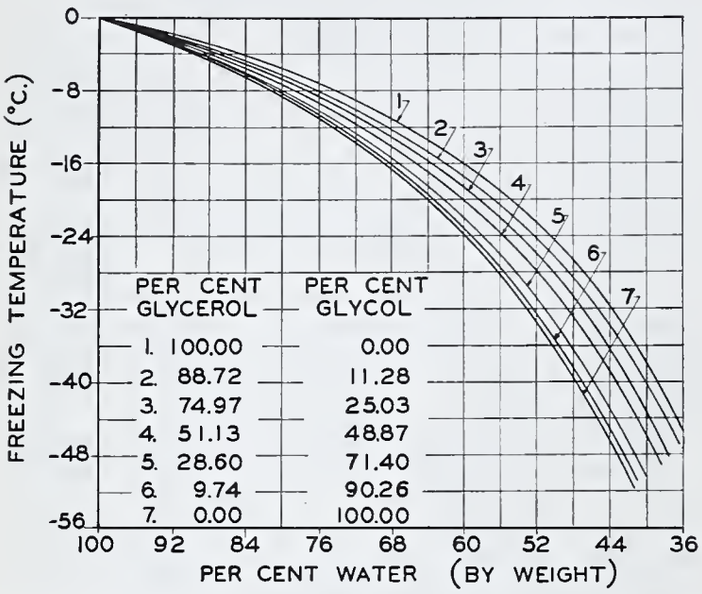

FIGURE 1. FREEZING TEMPERATURES FOR BLENDS OF GLYCEROL–ETHYLENE GLYCOL IN WATER

SINCE glycerol and ethylene glycol are both used extensively in the automobile antifreeze industry, the freezing points of aqueous mixtures of these compounds should be of interest. The following material gives the freezing points of mixtures of these three compounds, and an easy and exact method of analysis of any mixture of them.

Preparation of Solutions

The ethylene glycol was furnished by the Carbide and Carbon Chemicals Corporation and was purified by distillation at a reduced pressure of 40 mm. A fractionating column 45 cm. (18 inches) in length, packed with 7.5-cm. (3-inch) lengths of glass tubing, was used; and all connections were made with ground-glass joints. Only the constant-boiling middle portion of the distillate was retained, and the purified product showed at 25° C. an absolute density of 1.1101 and a refractive index of 1.4300.

TABLE I. FREEZING POINTS OF AQUEOUS ETHYLENE GLYCOL SOLUTIONS (° C.)

% by Weight of Ethylene Glycol	Curme and Young (*3*)	Bureau of Standards (*5*)	Olsen, Brunjes, and Olsen (*10*)	Conrad, Hill, and Ballman (*2*)	Present Work
10	−2.5	−2.9	−3.3	−3.6	−3.71
20	−7.7	−9.7	−7.8	−8.3	−8.45
30	−13.9	−17.6	−13.5	−14.4	−14.91
40	−23.3	−26.0	−22.1	−22.6	−24.05
50	−33.9	−37.0	−35.4	−34.6	−36.11

The glycerol used was Baker's analyzed, C. P. material. It was purified (from water) by double distillation at a reduced pressure of 3 mm. Only 20-cm. (8-inch) fractionating column was used in order to prevent decomposition by superheating. Bumping was effectively eliminated in both these distillations by filling the distilling flask to the level of the liquid with coarse-fiber Pyrex brand glass wool, and using a large distilling flask (3 liters) heated uniformly by an oil bath. The purified glycerol showed at 25° C. an absolute density of 1.2580 and a refractive index of 1.4720. Comparison with the density tables given in the International Critical Tables (*6*) and the Bosart and Snoddy tables (*1*) showed this glycerol to be 100 per cent pure. The glycerol and ethylene glycol were kept in 2-liter Erlenmeyer flasks, equipped with soda-lime tubes and siphon tubes. The liquid was forced out through the siphon tube by means of dried air and was thus exposed to undried air for only a very short time.

Ethylene glycol–glycerol mixtures were prepared by weighing to the closest milligram varying amounts of glycerol (10 to 125 grams) from

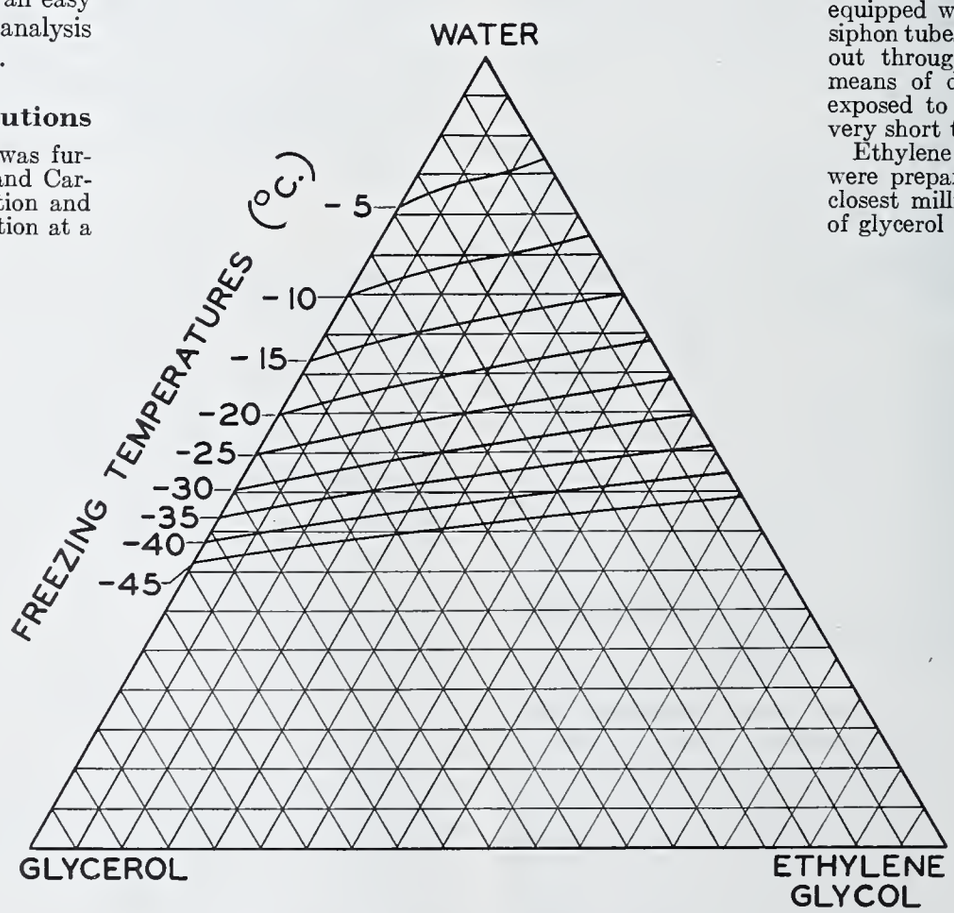

FIGURE 2. FREEZING POINT ISOTHERMS

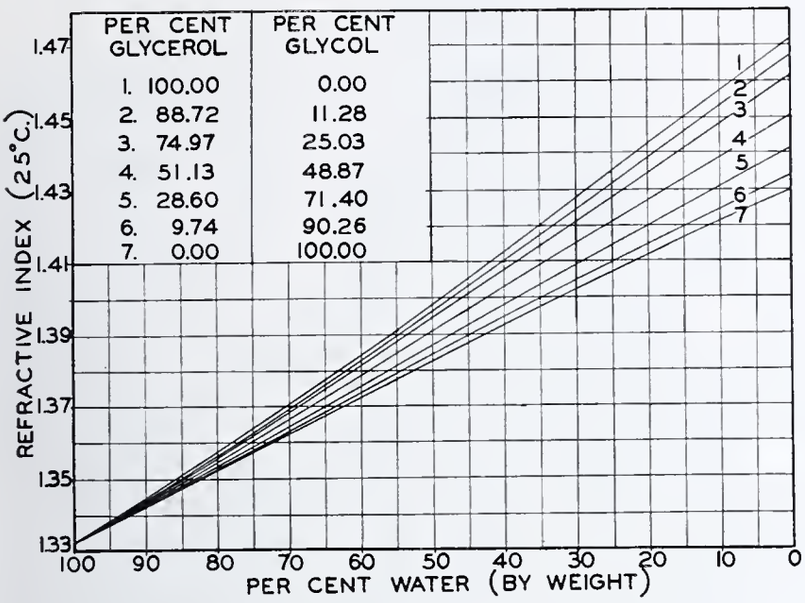

FIGURE 3. REFRACTIVE INDEXES AT 25° C. FOR BLENDS OF GLYCEROL–ETHYLENE GLYCOL IN WATER

TABLE II. FREEZING POINTS OF AQUEOUS GLYCEROL SOLUTIONS (° C.)

% by Weight of Glycerol	Lane (*8*)	Bureau of Standards (*5*)	Olsen, Brunjes, and Olsen (*10*)	Feldman and Dahlstrom (*4*)	Present Work
10	−1.6	−1.7	−2.3	−1.9	−1.99
20	−4.8	−4.8	−5.5	−5.4	−5.21
30	−9.5	−9.4	−9.8	−9.7	−9.92
35	−12.2	−12.3	−12.4	..	−12.65
40	−15.4	−15.6	−15.7	−15.6	−15.93
45	−18.8	−19.4	−18.6	..	−19.90
50	−23.0	−25.8	−23.8	−23.6	−24.55
55	−28.2	..	..	..	−30.40
60	−34.7	..	−37.2	−35.5	−37.90

a weighing buret. Ethylene glycol was then added in a similar manner to give solutions having approximate ratios of glycol to glycerol of 9 to 1, 7.5 to 2.5, 5 to 5, 2.5 to 7.5, and 1 to 9. Quantities of these blends were then weighed into ground-glass stoppered Erlenmeyer flasks and definite weights of water added from a standard, calibrated buret to make solutions containing approximately 10, 30, 50, 70, and 90 per cent of each blend. For the freezing point curves, it was necessary to weigh out two more samples for each blend at approximately 60 per cent blend in order to establish the eutectic point.

Freezing Point Determination

The freezing point apparatus used in this work consisted essentially of a 200-junction, copper-constantan thermocouple (one hundred junctions at each end), one end immersed in an ice bath and the other end in the solution, the freezing point of which was being determined. Provision for attaining equilibrium at each end was made by appropriate means of stirring, and the voltage developed was measured by means of a Leeds & Northrup Type K potentiometer and a Leeds & Northrup Type C galvanometer. The use of a thermocouple containing this number of junctions permitted an accuracy well within the limits of errors of the calibration values, using equipment generally available in most chemical laboratories. The thermocouple was calibrated by means of the following highly purified substances: water (0° C.), aniline (−5.98° + C.), carbon tetrachloride (−22.85° C.), chlorobenzene (−45.20° C.), and chloroform (−63.45° C.). The accuracy of these values vary from ±0.04° at 0° to ±0.06° at −65.0° C. The liquids being studied were cooled by the use of a bath of solid carbon dioxide and ethyl alcohol, separated from the freezing point tube containing the liquid by means of a Dewar flask. For the lower freezing points this Dewar flask was replaced by an ordinary freezing point tube, thus furnishing an air jacket.

Both cooling and heating curves were run, and three check determinations were made on both the freezing point and the melting point. The exact freezing points and melting points were obtained by extrapolation in the manner explained by Mair, Glasgow, and Rossini (*9*). The freezing point values differed from the melting point values by 0.02° to 0.05°, the latter figure applying to the lower freezing points.

Freezing Point Data

Tables I and II compare the values obtained in the present work with the freezing points obtained by other investigators for aqueous solutions of ethylene glycol and aqueous solutions of glycerol.

The values obtained in the present work are in good agreement with the average of the values obtained by the other investigators. The values for the lower temperatures should be much more accurate than the values obtained by the other investigators, because visual observation does not enter into the determination of the freezing point with the apparatus used in the present work. The increased accuracy due to this independence from visual observation is due to the fact that mixtures of ethylene glycol and glycerol with water get extremely viscous at low tem-

TABLE III. FREEZING POINTS OF ETHYLENE GLYCOL–GLYCEROL BLENDS IN AQUEOUS SOLUTIONS

Blend: Ethylene glycol	Blend: Glycerol	Freezing Points for the Following % by Weight of Total Blend in Aqueous Solution: 10	20	30	40	50	60
% by weight		*° C.*	*° C.*	*° C.*	*° C.*	*° C.*	*° C.*
100.00	0.00	−3.71	−8.45	−14.91	−24.05	−36.11	...
90.26	9.74	−3.54	−8.26	−14.57	−23.46	−35.35	...
71.40	28.60	−3.24	−7.71	−13.83	−22.21	−33.59	−49.84
48.87	51.13	−2.99	−7.10	−12.80	−20.50	−30.71	−46.12
25.03	74.97	−2.81	−6.64	−11.72	−18.59	−28.05	−43.29
11.28	88.72	−2.57	−6.18	−10.88	−17.20	−26.09	−40.45
0.00	100.00	−1.99	−5.21	−9.92	−15.93	−24.55	−37.90

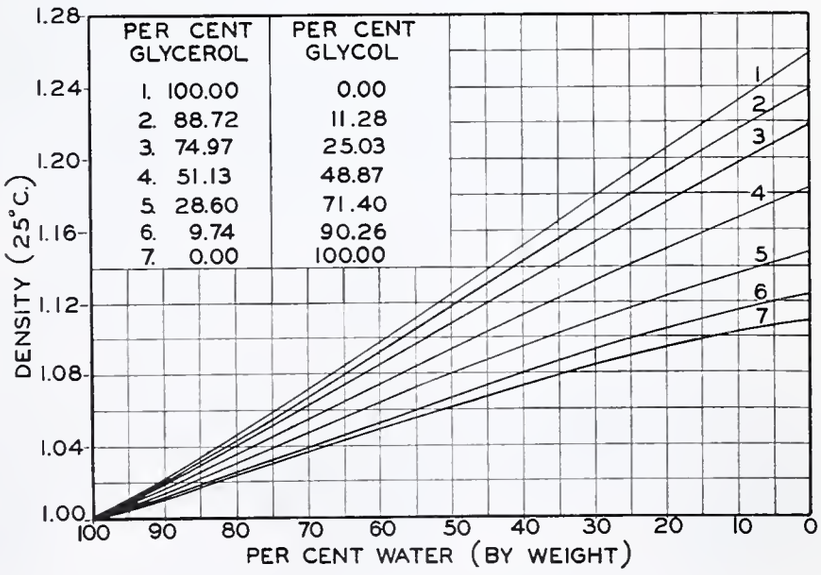

FIGURE 4. DENSITIES AT 25° C. FOR BLENDS OF GLYCEROL–ETHYLENE GLYCOL IN WATER

peratures and tend to trap air bubbles during stirring; this causes the solution to take on a milky appearance, making it very difficult to determine whether any crystals are present or not.

Figure 1 shows the curves obtained by plotting the freezing point against the per cent of total blend in aqueous solution. From these curves, values were taken for plotting isothermal curves on a triangular coordinate for every 5° as shown in Figure 2.

Table III lists the freezing points for compositions covering practically the whole range investigated. These data were obtained from the freezing point–composition curves.

Density and Refractive Index Data

The refractive indexes and absolute densities (all weighings reduced to vacuum and water of maximum density taken as unity) were determined at 25° C. using an Abbe refractometer and Leach pycnometers. Figures 3 and 4 show the curves obtained by plotting, respectively, the refractive index and density against the per cent of total blend in aqueous solution. From these curves, values were taken for plotting the constant refractive index and the constant density curves on the triangular coordinates shown in Figures 5 and 6.

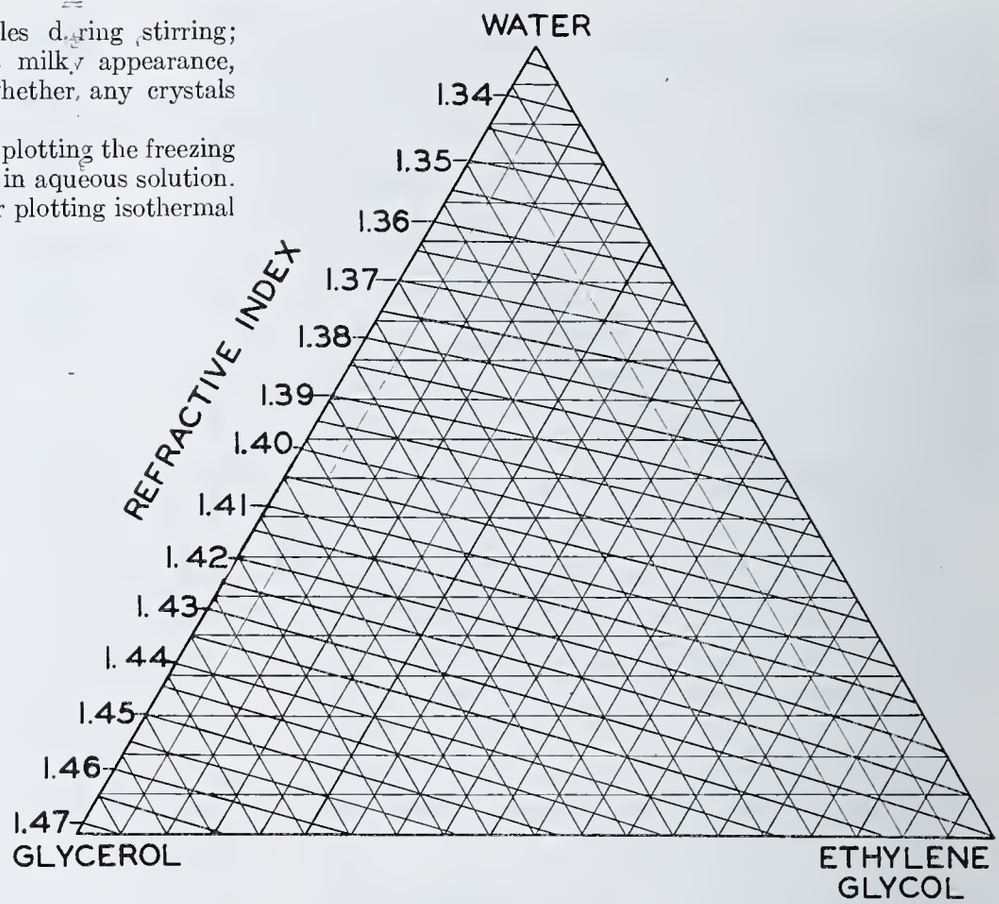

Figure 5. Constant Refractive Index Curves at 25° C.

Tables IV and V list the refractive indexes and densities for compositions covering the whole range of this system. These data were obtained from the refractive index–composition and the density-composition curves.

Figure 7 shows both the constant density curves and the constant refractive index curves. These curves intersect each other at an angle of approximately 30°. This makes it possible to determine accurately the amount of glycerol and ethylene glycol in an aqueous solution by merely obtaining the density and refractive index for that solution. The point of intersection of the density and refractive index curves for that solution gives the composition immediately. This method of analysis is more convenient, easy, and exact than any chemical means hitherto developed, and should be of great value in determining the ethylene glycol content (as an impurity) in glycerol, for the hygroscopic nature of both of these substances makes it a ternary system.

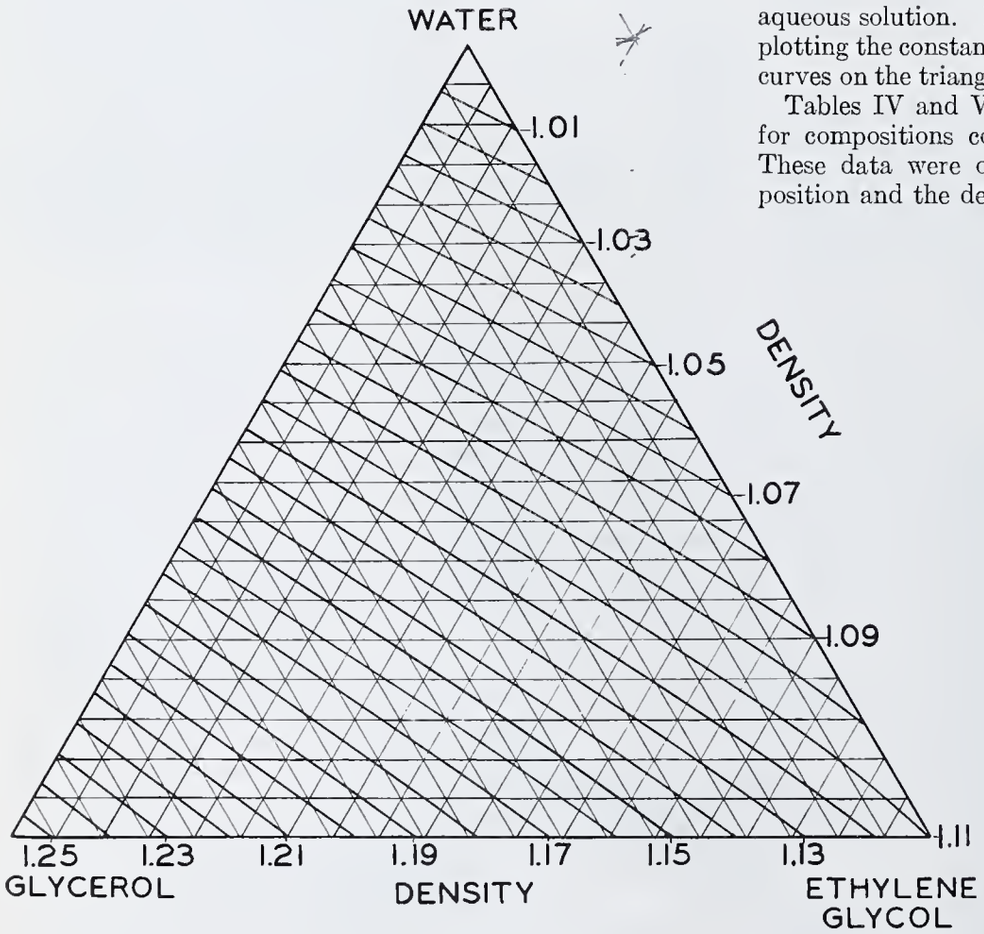

Figure 6. Constant Density Curves at 25° C.

Table IV. Refractive Indexes of Ethylene Glycol–Glycerol Blends in Aqueous Solutions at 25° C.

Blend: Ethylene glycol	Blend: Glycerol	Refractive Indexes for the Following % by Weight of Total Blend in Aqueous Solution: 10	20	30	40	50	60	70	80	90	100
% by weight											
100.00	0.00	1.3416	1.3517	1.3621	1.3723	1.3828	1.3927	1.4025	1.4118	1.4210	1.4300
90.26	9.74	1.3420	1.3522	1.3628	1.3736	1.3843	1.3946	1.4045	1.4148	1.4245	1.4343
71.40	28.60	1.3425	1.3533	1.3643	1.3757	1.3871	1.3981	1.4092	1.4202	1.4311	1.4420
48.87	51.13	1.3432	1.3547	1.3666	1.3786	1.3904	1.4026	1.4147	1.4269	1.4393	1.4516
25.03	74.97	1.3438	1.3558	1.3683	1.3809	1.3939	1.4073	1.4209	1.4343	1.4482	1.4624
11.28	88.72	1.3442	1.3563	1.3693	1.3826	1.3960	1.4100	1.4243	1.4388	1.4529	1.4670
0.00	100.00[a]	1.3447	1.3569	1.3701	1.3838	1.3977	1.4123	1.4273	1.4427	1.4568	1.4722

[a] Refractive indexes for glycerol taken from Iyer and Usher (*7*).

Table V. Densities of Ethylene Glycol–Glycerol Blends in Aqueous Solutions at 25° C.

Blend: Ethylene glycol	Blend: Glycerol	Densities for the Following % by Weight of Total Blend in Aqueous Solution: 10	20	30	40	50	60	70	80	90	100
% by weight											
100.00	0.00	1.0097	1.0231	1.0367	1.0495	1.0619	1.0733	1.0848	1.0946	1.1032	1.1101
90.26	9.74	1.0111	1.0259	1.0405	1.0544	1.0682	1.0813	1.0937	1.1051	1.1157	1.1250
71.40	28.60	1.0128	1.0300	1.0469	1.0631	1.0791	1.0952	1.1100	1.1239	1.1379	1.1521
48.87	51.13	1.0150	1.0349	1.0541	1.0738	1.0929	1.1110	1.1289	1.1478	1.1670	1.1832
25.03	74.97	1.0172	1.0406	1.0629	1.0857	1.1078	1.1302	1.1527	1.1755	1.1976	1.2187
11.28	88.72	1.0193	1.0438	1.0674	1.0921	1.1165	1.1411	1.1666	1.1918	1.2161	1.2399
0.00	100.00[a]	1.0207	1.0453	1.0707	1.0971	1.1238	1.1511	1.1784	1.2055	1.2320	1.2580

[a] Densities for glycerol taken from Bosart and Snoddy tables (*1*).

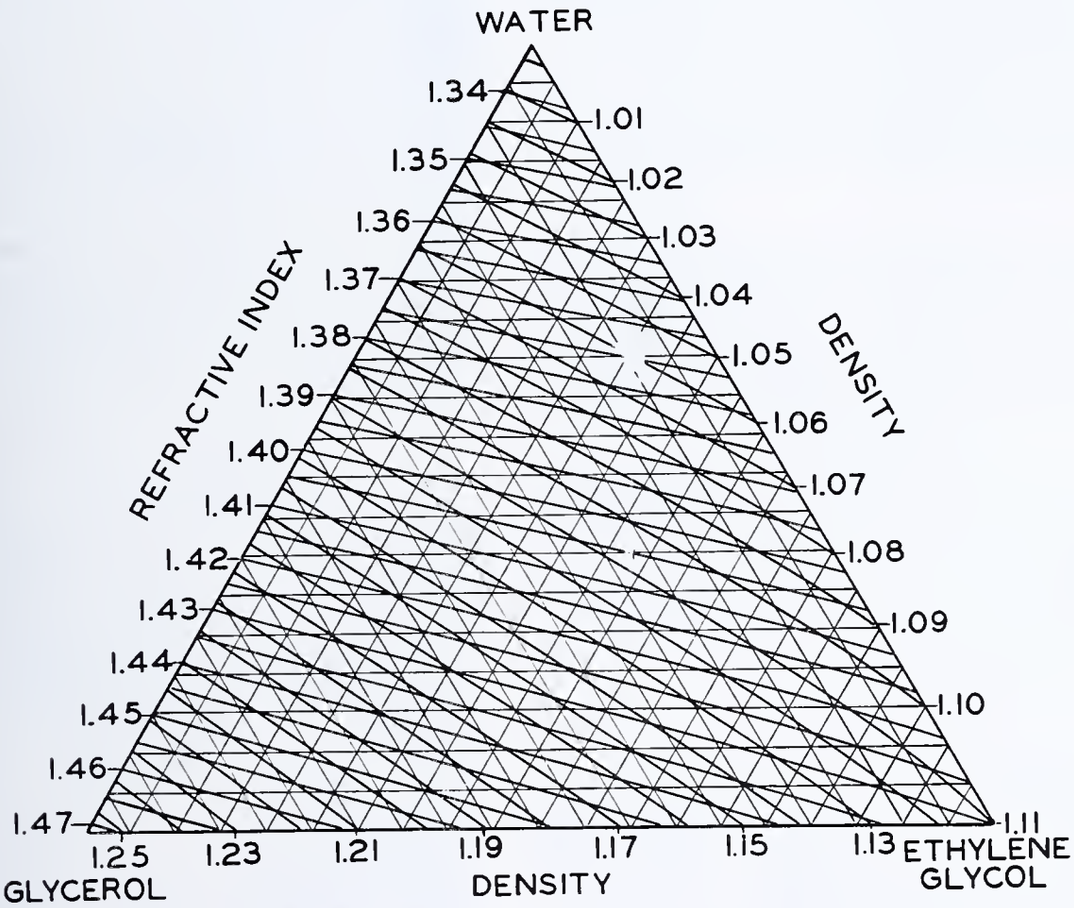

Figure 7. Constant Density and Refractive Index Curves at 25° C.

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Determination of Thiamine in Bread by the Thiochrome Method

A Comparison of Phosphatase-Containing Enzyme Preparations

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ANDREWS and Nordgren (*1*) have suggested that a great deal of the difficulty experienced in assaying bread for thiamine by the thiochrome method may be due to the presence of cocarboxylase formed from the free thiamine by the action of yeast.

These authors raised the incubation temperature and lengthened the incubation time in order to accomplish complete hydrolysis of cocarboxylase. Lipton and Elvehjem (*8*) have shown that thiamine can be coupled with phosphate by yeast to give cocarboxylase, and Livshits (*9*) found that yeast may be capable of synthesizing thiamine.

Conner and Straub (*3*) and Hennessy, Tarshis, and Perlman (*5*) have shown that several commercial enzyme preparations will quantitatively hydrolyze aqueous solutions of synthetic cocarboxylase. The latter authors also found aluminum, mercuric, and ferric ions inhibiting, ferrous accelerating, and considerable variation in the enzymes. It is well known that optimum concentrations of accelerators are necessary for many phosphatases if they are to operate at maximum velocity.

Besides commercial enzyme preparations, which are usually made from mold growths, other phosphatase preparations have been used to hydrolyze cocarboxylase: Hennessy and Cerecedo (*4*) used a beef kidney extract, Kirch and Bergeim (*7*) used a yeast-glycerol extract, and Melnick and Field (*10*) used a yeast powder.

In view of these investigations it appeared possible that solutions of pure cocarboxylase might not give the same results with phosphatase preparations as would cocarboxylase in natural products with phosphatase preparations, because the work done with pure cocarboxylase does not take into account the effect of numerous activators and inhibitors that might become active when the cocarboxylase in natural products is to be hydrolyzed.

The authors were interested in finding an enzyme preparation that would efficiently hydrolyze the cocarboxylase in bread in a short time and under the conditions of pH and temperature of digestion of the thiochrome method as it is generally prescribed. This enzyme was preferably to be either a readily available commercial preparation or one that is easily prepared in the control laboratory.

Procedure

Four commercial enzyme preparations and three preparations made in this laboratory were tested, using the thiochrome reaction as an indicator of the power of the enzyme to split cocarboxylase. A considerable range of results was found, and, as was to be expected, the results on bread did not coincide with those on aqueous solutions of pure cocarboxylase. Variations were also noticed in the results obtained with different kinds of bread. The assay procedure was that of Conner and Straub (*3*) with minor modifications. The instrument used for the final readings was a Coleman Model 12 electronic photofluorometer. The enzyme preparations were as follows:

Enzyme	Source
Takadiastase	Parke, Davis & Co.
Clarase	Takamine Laboratory, Inc.
Mylase P	Wallerstein Laboratories
Polidase	Schwarz Laboratories, Inc.
Aspergillus flavus	Grown in this laboratory
Yeast-glycerol extract	Prepared in this laboratory after Kirch and Bergeim (*7*)
$La(OH)_3$ solution	Used in water solution. Suggested by the work of Bamann and Meisenheimer (*2*), who found that $La(OH)_3$ cleaves sodium glyceryl-β-phosphate

TABLE I. ASSAYS OF BREADS

Enzyme[a]	Bread	Incubation Time[b] *Hours*	Thiamine *Micrograms/g.*
Takadiastase	Commercial 1	16.5	3.11
Takadiastase	(sponge process)	1.5	2.55
Clarase		16.5	3.09
Clarase		1.5	2.52
Clarase		20	3.15
Clarase		18	3.08
Polidase		20	3.10
Polidase		1.5	3.28
Polidase		18	3.01
No enzyme (blank determination)		1.5	1.62
Takadiastase	Commercial 2	1.5	3.51
Clarase		1.5	3.03
Polidase		1.5	4.35
No enzyme (blank determination)		1.5	1.41
Takadiastase	Commercial 3	1.5	3.96
Clarase		1.5	3.42
Polidase		1.5	5.04
Takadiastase	Commercial 4	1.5	1.32
Clarase		1.5	1.32
Polidase		1.5	1.71
Clarase	Commercial 5	16	3.12
Polidase		1.5	3.33
Takadiastase	Laboratory bread enriched with yeast	1.5	1.02
Clarase		1.5	1.35
Polidase		1.5	2.40
Mylase P		1.5	2.13
Yeast extract		1.5	1.14
Takadiastase	Laboratory bread not enriched	1.5	0.98
Clarase		1.5	0.78
Polidase		1.5	1.20
Mylase P		1.5	0.99
Yeast extract		1.5	0.51

[a] Assays of 1-gram samples of enzymes indicated that they were essentially thiamine-free.
[b] Moisture loss was found negligible with long incubation, and no correction was necessary.

The *Aspergillus flavus* was grown on a 1 per cent peptone–4.5 per cent sugar–tap water media, dried by an air blast at room temperature, and ground up. It was stored out of direct light at room temperature, as were the commercial enzymes. The yeast extract was kept under refrigeration, where it is stable for months (*6*). The lanthanum hydroxide did not give encouraging results and was abandoned early in the investigation.

The bread samples were broken into small pieces and air-dried to about 10 per cent moisture, then ground until 99 per cent passed through a 36 grits gauze, and blended carefully. They were stored at room temperature out of direct light. Four-gram samples were taken for assay, and the results are given on the "as run" moisture basis. Most of the work was done with a commercial bread (commercial bread 1) made by the sponge process and enriched with a concentrate containing iron, thiamine, and nicotinic acid, as well as a yeast food. Such yeast foods usually contain sodium chloride, calcium sulfate, ammonium chloride, and potassium bromate. Table I summarizes the results of 68 assays on this bread and 21 assays on six other assorted breads. The figures given represent averages of results, all of which agree within 5 per cent.

It is apparent here that the polidase gives the best results, whether the time is 1.5 hours or overnight, with mylase P giving second best. For commercial bread 1 mylase P and the yeast preparation did quite as well as polidase, as will be seen in Figure 1. In all cases takadiastase and clarase gave quantitative results only after incubation periods of many hours.

Using the sponge process bread (commercial bread 1) time curves were run on all the enzyme preparations. The results

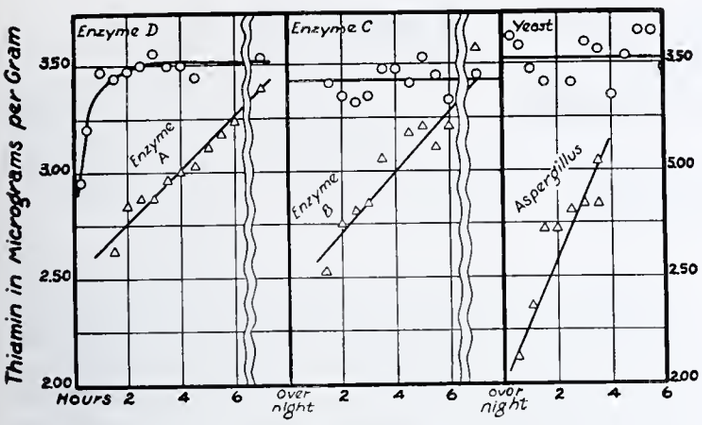

FIGURE 1. TIME CURVES PRODUCED BY ENZYME PREPARATIONS ON SPONGE PROCESS COMMERCIAL BREAD 1

All preparations were 3% suspensions in the buffer, except the mold, which was 1.1%. Temperature, 45°–50° C. *A*, takadiastase; *B*, clarase; *C*, mylase P; *D*, polidase

are shown in the series of small graphs in Figure 1. The curves were continued until further hydrolysis gave no more thiamine, with one exception.

It is evident from these graphs that only polidase, mylase P, and the yeast extract gave complete hydrolysis in 1.5 hours, and since the yeast extract and mylase P failed to give quantitative results on the laboratory breads (see Table I) it follows that only polidase can be depended upon to give good results in 1.5 hours on all the breads tested. It cannot be predicted that it would likewise give quantitative results on any bread tested. The *Aspergillus flavus* preparation followed closely the curves set by takadiastase and clarase. Since it is difficult to prepare, no further work was done with it.

A concentration curve was run, using polidase and commercial bread 1 (Figure 2). It is apparent from this graph that it is inadvisable to use concentrations of polidase below 3 per cent in the buffer when the incubation time is 1.5 hours and the temperature 45° to 50° C.

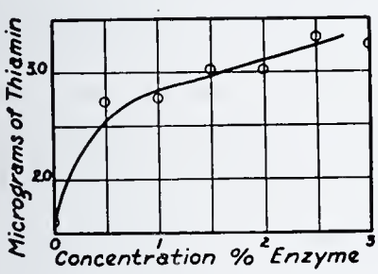

FIGURE 2. CONCENTRATION CURVE WITH POLIDASE

Incubation time 1.5 hours, temperature 45° to 50° C.

To compare the action of the various enzyme preparations on aqueous solutions of pure synthetic cocarboxylase, a series of experiments was undertaken using water solutions of cocarboxylase made 0.04 *N* with sulfuric acid. The buffer solution of enzyme was added as usual. Some 125 assays revealed such variations that no attempt was made to draw any definite conclusions as to the relative merits of the enzymes. Some typical results are shown in Table II. Only polidase gave quantitative recovery consistently. The causes for this, while unknown are assumed to be inhibitors and accelerators that appear in varying amounts as contaminants in the apparatus.

It seems reasonable to conclude from these experiments that variable results with enzymes used to hydrolyze cocarboxylase in bread—for example, the variation in the results obtained with mylase P and the yeast extract—may be attributed to the differences existing between various kinds of enzymes, and perhaps also to the presence of varying amounts of activators and inhibitors in different breads. It also seems reasonable to assume that experiments with aqueous solutions of pure cocarboxylase may not give the same results as with cocarboxylase in bread. It is recommended that, in using any enzyme preparation, the enzyme be allowed to incubate for at least 18 hours in order to ascertain the shortest incubation time possible with that particular enzyme and bread.

Summary

An enzyme was found that gave complete hydrolysis in 1.5 hours on all breads tested when they were assayed for thiamine content by the thiochrome method. Other enzymes gave results that varied considerably with the kind of bread used. When used with solutions of pure cocarboxylase, all but one of the enzymes gave results inconsistent with those obtained when they were used on bread. These variations are assumed to be due to inherent differences between various kinds of enzymes and to the presence of varying amounts of activators and inhibitors in different breads.

TABLE II. HYDROLYSIS OF AQUEOUS SOLUTIONS OF COCARBOXYLASE

(Containing 10.0 and 12.5 μg. of cocarboxylase per 50-ml. aliquot)

Enzyme	Incubation Time	Recovery
	Hours	%
Polidase	0.25	100.0
Polidase	2	100.0
Polidase	3	100.0
Takadiastase	2	2.5
Takadiastase	2	50.0
Takadiastase	3	10.0
Clarase	2	100.0
Clarase	2	10.0
Mylase P	2	100.0
Mylase P	2	60.0
Yeast extract	0.50	100.0
Yeast extract	1.5	78.0
Yeast extract	2	10.0
Blank (no enzyme)	1.5	0.0

Acknowledgment

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Quantitative Spectrographic Analysis of Stainless Steels

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A spectrographic method of analysis is described that allows rapid and accurate quantitative analyses of all metallic constituents in stainless steel stock. A direct current arc and a specially shaped carbon electrode allow chromium and nickel analyses accurate to 2 per cent of the quantity being measured. At the same time, satisfactory silicon, manganese, columbium, molybdenum, and titanium analyses can also be made. Complete analytical details are furnished, including a method of calculating results that takes into consideration the wide variation in iron content likely to be experienced in this type of material.

IN THE last five years the spectrograph has been applied successfully to the analysis of cast iron and low alloy steels (*1*, *3*). In these fields the great economic advantage of a method so rapid and accurate that it can give analysis in the foundry before the metal is cast, and so convenient that one analyst can do the work of five, was bound to be appreciated. With the advent of the war, such advantages are even more valuable. However, the great need at the moment is to extend these methods to more and more alloys, so that testing methods can be made to keep step with the tremendous increase of metal production that is being experienced at this time.

The authors' laboratories have attempted to contribute to that need by developing a method of stainless steel analysis that is sufficiently accurate and rapid to be used for the routine analysis of chromium, nickel, manganese, silicon, columbium, molybdenum, titanium, and any other significant element by a single spectrographic procedure. The method represents a distinct step forward, as it allows the analysis of chromium up to 28 per cent and of nickel up to 20 per cent with results precise to ±2 per cent of the quantity being measured. This produces analyses, such as chromium at 28 ± 0.56 per cent, at 18 ± 0.36 per cent, and at 12 ± 0.24 per cent; and nickel at 20 ± 0.4 per cent, at 12 ± 0.24 per cent, and at 8 ± 0.16 per cent. At first glance, this accuracy may not appear as acceptable as chemical methods, but a careful comparison, using repeat chemical analyses by different laboratories and spectrographic analyses by different operators, indicated that the latter suffer much less from random accidental errors than the former, and hence should be just as acceptable, particularly for routine testing. When the determinations of minor constituents are compared, the spectrographic method proves more accurate than the chemical method. Thus the combination of acceptable consistent analyses at the higher percentages, and very precise analyses at the lower percentages for a number of the metallic elements in stainless steel, makes this method of distinct value.

In addition to extending the spectrographic method to the determination of alloying constituents at high percentages, this work, in providing for the analysis of ordinary sheet and wire stock, removes the limitation of specially produced samples which has been found necessary in much previous work of comparable accuracy.

Sampling of Alloys

In connection with the latter point, it will be of advantage to discuss the sampling problem of alloys with special reference to spectrographic work. This problem is related to the particular light source used to produce the spectrum of the sample, depending upon whether it is an electric spark or an arc. The spark is characterized by its repetitiousness and very small sample consumption, the direct current arc by its continuousness and relatively large and complete sample consumption. Numerous authors have characterized the spark as being highly reproducible and hence most suitable for accurate quantitative analysis, and the direct current arc as being nonreproducible and hence suited only for qualitative or semiquantitative work. That this last conclusion is not necessarily true and that the sampling problem can be solved to a considerable extent by employing an arc is the basic premise of the method to be presented.

In the case of the spark, either 60 or 120 electrical strokes per second are passed between electrodes of the material to be analyzed. Exposures from 10 to 60 seconds give thousands of strokes which play about the top of the sample exposed and thus thoroughly sample the surface presented for analysis. It is this constant repetition of sparking that gives an aggregate exposure that is highly reproducible, providing the surface presented to the discharge is in turn highly reproducible, both as to composition and physical state. This reproducibility of composition and physical state may in some cases be very important, as the spark often tends to etch the surface exposed by direct evaporation rather than by first melting and then evaporating. It thus is selective in its action and only by careful production of the surface to be sparked can assured results be obtained for all types of alloys.

To make certain that at least the composition of the surface exposed to the spark is representative of the sample, numerous methods of sampling have been worked out. The most convenient one is to take drillings or filings from various points on the sample and briquet them into an electrode. This furnishes a composite surface for sparking, which, as a whole, represents the average analysis of the sample—is average in composition. However, this procedure does not ensure a reproducible physical state on the sparking surface, irrespective of the sample's metallurgical history. That it has been highly successful in many instances indicates that for certain types of materials, such as the low percentage alloys, for instance, the physical state is well reproduced by ordinary metallurgical practice.

However, for high percentage alloys, such as stainless steel, produced under a variety of metallurgical conditions, no method of sampling, short of dissolving the metal completely or melting it under controlled conditions, would be expected to produce a sample representative in composition and uniform in physical form. The fact that certain of the elements

determined may be in some cases combined with the major constituents, while in other cases they may be present in grain boundaries as carbides or oxides, certainly indicates that traditional spark methods are likely to give very uncertain results of this type of material.

That this is actually the case has been proved by attempting to analyze 0.3-cm. (0.125-inch) stainless steel wire stock with a commercially available, controlled high-voltage spark unit. Sparking between two machined ends of wire pieces and using sparking conditions that give very accurate analyses on low percentage alloy steels, extremely erratic results were obtained. Groups of eight repeat analyses, on three samples, using remachined wire surfaces, gave an average deviation for the chromium to iron intensity ratio of 10.1 per cent, a nickel to iron 4.8 per cent and manganese to iron 5.4 per cent. Silicon, at approximately 0.5 per cent, could not be measured at all under these discharge conditions. This is good evidence that the composition of these wires is not sufficiently uniform for direct spark analysis. If briquetting were to be used, the average results obtained from the eight runs of each sample would be obtained with less random error. However, when these averaged results were plotted against percentage composition, no consistent relationship appeared, proving that the physical form of the alloys was not sufficiently consistent to enable direct spark analysis of either rod or briquetted samples.

Method of Sampling and Arcing

It was with these facts clearly in mind that a method of sampling and arcing was developed that allows accurate analysis of material, irrespective of its metallurgical history.

The basic idea is very simple. If a fine file is used, filings averaging 0.04 mm. in diameter can be easily obtained from sheet or wire stock. These can be taken from a number of portions of the piece to be tested and thoroughly mixed to produce a sample of good average composition. A 3-mg. portion of these filings contains about 1500 particles, which should be an adequate number to provide a good average composition. This conclusion is borne out by the reproducibility obtained upon duplicate analysis. This 3-mg. portion is placed upon a special carbon electrode and cemented in place with a drop of sugar solution which has been preceded by a drop of alcohol to wet the carbon. The surface tension of the sugar solution spreads the stainless steel particles over the surface of the electrode where they are deposited upon drying.

Figure 1 shows the type of special electrodes used for both the top and bottom electrodes of the direct current arc. Upon arcing, the discharge starts at the center post above the sample and holds to it for about 30 seconds. This heats the sample sufficiently by conduction, so that it melts first and then evaporates up into the arc. The melting erases the history of the solid sample and allows a reproducible state of affairs. The arcing proceeds smoothly as the molten metal stays spread out in a thin layer covered with many small bumps. There is no tendency to form a single large bead of metal, which usually causes arc wandering and unstability of the discharge. Rather, the center post burns away smoothly, vaporizing much of the metal as it does. This is followed by vaporization of the conical platform that carries the sample. When nothing remains but the stub, one is assured that every bit of the sample has been evaporated. This arcing to completion assures accurate results for the determination of refractory substances used as stabilizers, such as compounds of titanium and columbium, as well as for the more volatile metals.

With the sampling problem solved, the next question is, will such an arc give reproducible results; will the intensities of certain chromium, nickel, silicon, manganese, etc., lines in the spectrum bear a certain reproducible relationship to certain iron lines in the spectrum? To answer this question, it is necessary to determine first which spectrum lines are most suited to the purpose. The usual procedure is to vary such things as arc current, distance between electrodes, etc., and determine which line ratios of intensity remain the most constant for these changes. However, in this case, since these quantities could be controlled very accurately, it appeared best to study the variation in line intensity ratios as a function of the total amount of sample on the electrode. This was done, not because this quantity cannot be closely controlled, but rather because variations in this discharge will result mainly from differential vaporization effects, and change of discharge characteristics with time through the arcing cycle. By deliberately changing the basis for such effects, line pairs can be chosen that minimize the effect of possible variations.

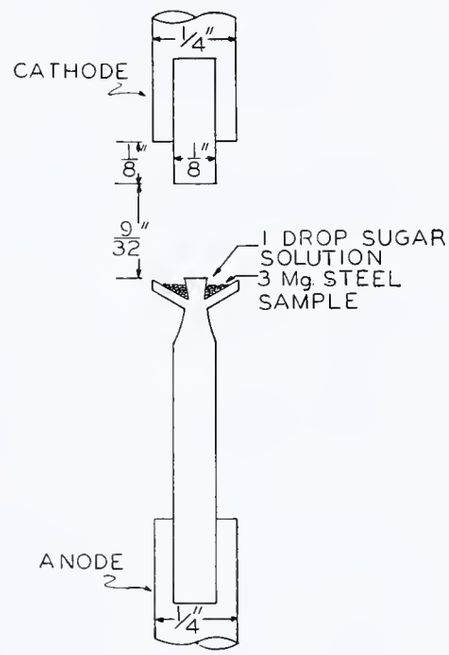

Figure 1. Special Carbon Electrodes Turned from Rod Stock

After making the final choice of line pairs, the whole problem of reproducibility was reviewed by running a number of arcings on a wide variety of samples to determine what the average variation in selected line intensity ratios would be. This was preferred to the customary procedure of repeating the process over and over again on the same sample, as in that case the properties of the particular sample used determine the results obtained to a large extent. If many samples are used, an average reproducibility can be determined that is significant for the entire practical problem on hand. The average deviations from the means obtained from 80 determinations of each element were as follows: chromium 2.5 per cent, nickel 2.6 per cent, silicon 2.8 per cent, manganese 3.2 per cent, all of the percentage measured.

This reproducibility is not quite so good as some that have been reported on specially prepared samples (*2*), but it is very good when the wide variety of samples used is considered. However, it still is not sufficient for the accurate determination of the high percentages desired. In general, there is a very simple way to improve analytical accuracy—that is, repeat the procedure and average the results. The gain in precision is equal to the square root of the number of determinations averaged to produce a single final determination. By averaging two arcings, the precision is thus increased 1.4 times. Two ways of handling this duplication are possible. One is to use two arcings to produce a single spectrogram, the other is to use each arcing to produce a single spectrogram. In the first case, one set of readings on the various line blacknesses with the densitometer gives the average result automatically, while in the second case two sets of readings are

Table I. Repeat Arcings

Sample analysis: chromium 18.7%; nickel 9.9%; silicon 0.46%; manganese 0.50%. Exposure: single without averaging two. Percentage deviation of analysis obtained by applying average percentage deviation to working curves of Figures 3 and 4. Deviations for two arcings obtained by averaging all possible pairs of results, calculating average deviations from these averages, and then applying these to working curves.

Intensity Ratios	1	2	3	4	5	6	7	8	Average	Average % Deviation	% Deviation of Analysis 1 arcing	% Deviation of Analysis 2 arcings
$\frac{I_{Cr}}{I_{Fe}}$	1.27	1.28	1.26	1.24	1.27	1.31	1.30	1.32	1.281	1.6	2.2	1.4
$\frac{I_{Ni}}{I_{Fe}}$	1.29	1.29	1.31	1.33	1.26	1.31	1.31	1.26	1.295	1.5	2.5	1.6
$\frac{I_{Si}}{I_{Fe}}$	0.87	0.90	0.94	0.88	0.87	0.94	0.91	0.89	0.900	2.3	2.8	1.8
$\frac{I_{Mn}}{I_{Fe}}$	0.69	0.71	0.70	0.66	0.65	0.70	0.69	0.65	0.681	3.1	3.5	2.1

required and the results averaged. Obviously the second method is more time-consuming, but it averages over all other errors as well as the arc errors, so that it produces results slightly more accurate than the first. With it, average deviation on repeat applications of the whole procedure—double arcing and double line reading—gave the following average deviations from the mean: chromium 1.8 per cent, nickel 1.8 per cent, silicon 2.0 per cent, manganese 2.3 per cent, all of the percentage measured.

These values are very acceptable, as they allow consistent work to be done within the final error indicated in the introductory remarks.

In order to show the precision to be expected on a short run, the results of eight repeat arcings are presented in Table I. These are single arcings and hence, used as such, should give errors similar to the first group of results presented. In view of the small number of runs utilized, the agreement with the over-all average appears about right. Taken as pairs and averaged, the results should correspond to the second group of results. Again the agreement appears proper, illustrating the gain in accuracy obtained by this procedure.

With a reproducible method of handling complex metallurgical samples devised, what other problems arise in high percentage analysis that are not present at low percentages? The main one is the internal standard problem. In low alloy steels, the intensity of each element's lines is compared to iron, and since iron varies only from 90 to 100 per cent, even in extreme cases, this comparison can be made without regard to the iron content of the sample. At first consideration, this may appear as poor practice if it is desired to measure the various alloying elements very accurately. Actually, the intensities of most iron lines change very little for a substantial percentage change in the region 90 to 100 per cent iron. Only because of this can the comparison be made without regard to the iron content. However, in stainless steel work, the iron content may vary from as low as 55 per cent to as high as 90 per cent. In this case, the percentage change of iron will cause an appreciable change in iron line intensities, which certainly cannot be ignored.

At first it appeared that the only way to solve this problem was to introduce another element which was not present in steels as an internal standard. Some work was done along this line, using gold. Although the results were promising, it was found possible to go back to iron as the internal standard if corrections for variations in the iron content of the samples were made.

With the two basic problems solved, means for preparing a reproducible record easily, and means of interpreting that record, the rest of the work proceeded along conventional lines. However, in order to make that work available to other workers in the field, the method and results obtained with it are described in detail.

Apparatus and Method

Sample Preparation. Use a No. 2 American-Swiss spark-plug file and obtain a total of approximately 100 mg. of filings from a number of different points on the sample. Sawed edges make good surfaces for filing, as then the body of the metal can be sampled. Collect these filings on a piece of paper, transfer them to a glass vial, and mix them thoroughly by stirring. Prepare a small scoop by drilling a conical hole on the side of a 0.31 × 0.16 cm. ($^1/_8 \times {}^1/_{16}$ inch) rectangular rod, close to one end. This hole is about 0.175 cm. (0.070 inch) in diameter and conical. It is produced with an ordinary machinist's drill. Adjust the depth of the hole so that 3 mg. of sample will just fill the hole when it is scooped out of the vial with the rod, tapped, and leveled with a spatula. This can be done with a few trials. With a little experience, an operator can fill such a scoop with 3 ± 0.15 mg. every time. This represents a possible error of 5 per cent in weighing. However, since the intensities of all lines of the elements to be measured are compared to iron line intensities, this does not introduce an appreciable error in the result. This is particularly true for the lines chosen, as these varied little in intensity ratio with a large change in the total amount of sample.

Electrode Preparation. All lower electrodes should be prearced 6 seconds after the current has been adjusted to 10 amperes on short circuit of the electrodes. This vaporizes the impurities common to graphite electrodes not of superpure grade that may interfere with certain of the determinations to be made, such as silicon and titanium. Besides this, the prearcing puts the electrode in a more porous form, so that the molten metal can more readily obtain intimate contact with it. A decided drop in reproducibility is noted if electrodes are not prearced, so that this represents an important point that should be given careful attention. Prearcing can be done for a group of lower electrodes just prior to their final preparation.

With the scoop, load 3 mg. of sample into each of two electrodes. This provides the duplicate electrodes necessary for very high precision. Tap the base of the electrode to distribute the sample around the center post. Place a drop of alcohol on the electrode to wet it, followed by a drop of sugar solution containing 4 mg. of sugar per drop. Gently heat the electrode over a flame until the sugar solution dries. Do not overheat, as carbonizing the sugar produces a variation in the starting condition of the electrodes.

Arcing Conditions. The electrodes are centered accurately on the optical axis by means of a projection system. Vertically they are arranged 0.352 cm. ($^9/_{64}$ inch) above and below the optical axis, giving a total electrode spacing of 0.703 cm. ($^9/_{32}$ inch) between electrodes. All measurements are made to the platform rather than the tip of the lower electrode. The arc is struck by moving the upper electrode down onto the lower, and then rapidly raising it until it reaches its original position, determined by a stop.

The arc current supplied by a commercial spectrographic rectifier unit is adjusted to 10 amperes with the electrodes short-circuited. Upon striking the arc, the current is about 7.5 amperes. It gradually decreases as the arc gap widens. No adjustment of the electrode positions or of the current is made while the arc is on.

The arc is allowed to vaporize sample and electrode until the entire platform is consumed and only the stub remains. This requires about 80 seconds.

The entire arcing is photographed to produce a single spectrogram.

Spectrograph Employed. The spectra of iron, chromium, and nickel are all very complex. Thus, it is essential to have a spectrograph of adequate resolution and dispersion for stainless steel analysis. The instrument utilized was a commercial grating spectrograph (A. R. L.-Dietert) having a high resolving power, 48,000, and adequate dispersion, 7 Å. per mm.

The instrument utilizes 35-mm. motion picture film, which provides the most uniform emulsion obtainable.

Illumination of Spectrograph Slit. The arc is placed 28 cm. beyond the height-limiting aperture of the astigmatic grating spectrograph, which is, in turn, 18 cm. beyond the

Table II. Spectrum Lines

Elements	Wave Length of Line	Transmission Readings (Typical Example)	Intensities Deduced from Figure 2
Chromium	2879.3	18.3	1.38
Nickel	2821.3	19.0	1.34
[a]	3101.6		
Manganese	2933.1	39.1	0.79
Silicon	2506.9	44.1	0.72
Columbium	2950.9	...	...
Molybdenum	2816.2	...	...

[a] For low percentage nickel alloys.

Table III. Spectrum Lines of Iron

Wave Length	Known Relative Intensity Values	Transmission (Typical Example)
2950.2	1.50	16.2
2872.3	1.00	28.3
2869.3	2.13	10.1
2840.4	1.08	25.9
2838.1	2.30	9.2
2828.8	0.76	41.2
2804.5	2.59	8.0

primary slit. No condensing lens is used. This setup of the astigmatic spectrograph gives spectra with sharply defined edges, such as are obtained with a stigmatic instrument.

Film Processing. To obtain the best results, the uniformity of film sensitivity must be matched with uniformity of development. This is accomplished with commercial apparatus made specifically for handling film under reproducible conditions of developer agitation and temperature. High-speed washing and drying allow complete film processing in 4 to 5 minutes.

Film Measurement. A projection comparator-densitometer provides a very simple method for finding the lines required and measuring their degree of blackness. The identification of lines is made by a linear direct-reading scale on a master plate which allows them to be located by their wave lengths. Their transmission values are then read with a high-precision photoelectric densitometer.

Spectrum Lines Employed. Table II gives the spectrum lines of the various elements used in making analyses. Table III gives the various spectrum lines of iron used, both as internal standards and for film calibration. These iron lines have been carefully chosen, so that they remain constant as to their relative values over large changes in iron content.

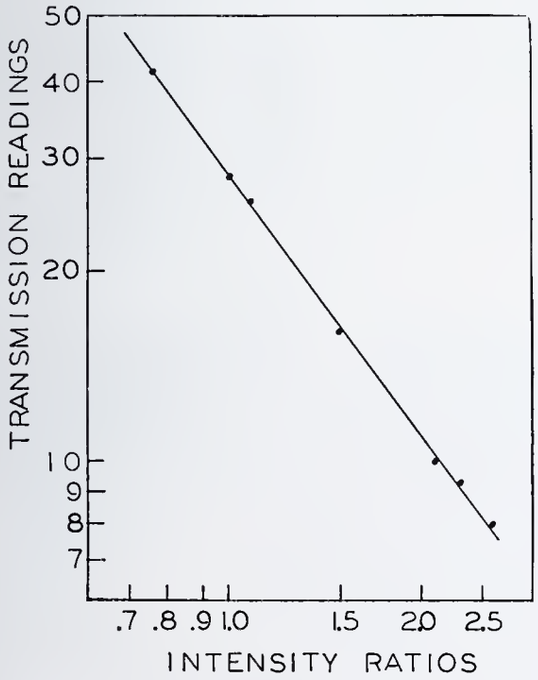

Figure 2. Typical Film Blackening Calibration Curve

Determined from lines of known relative intensity

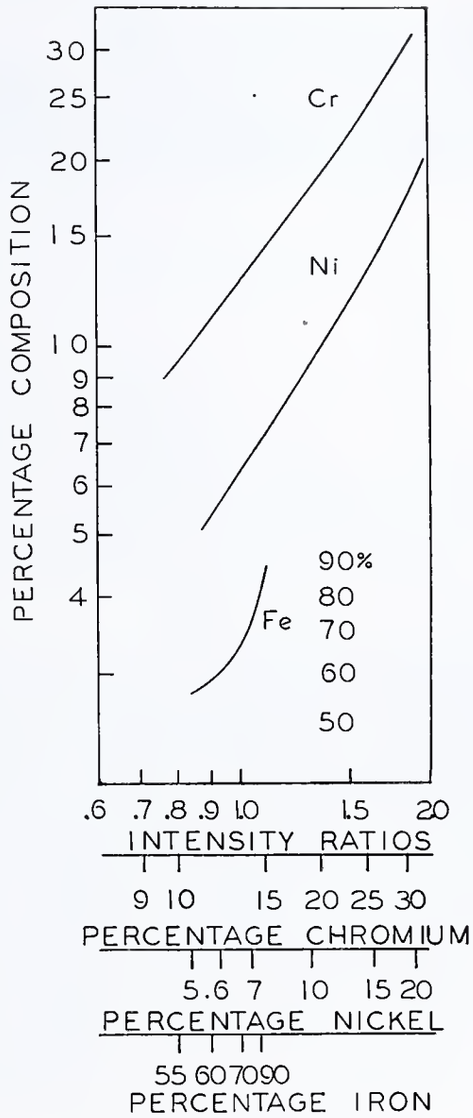

Figure 3. Working Curves and Scales for Chromium and Nickel Determinations

With unity point correction curve for iron content of sample

The "known values of intensity" have been determined by standard stepped-sector methods. They should be rechecked with the spectrographic equipment to be used, as variations in the exact positioning of the arc may cause differences.

Calculations

Calculation of Relative Intensity Values. This group of iron lines is read in each spectrogram and plotted as shown in Figure 2—log transmission as ordinate, log intensity as abscissa. This gives the usual film-blackening calibration curve inverted, by which differences in developing conditions, film contrast, etc., are eliminated from the results. Using this curve, the transmission readings of the lines of the elements to be determined are converted to relative intensity values, relative to the iron 2872 Å. line being considered as of intensity unity. These are given for the typical example shown.

This procedure is repeated for each spectrogram, so that each relative intensity is deduced from the best average curve through all the iron line values for that exposure. This is a

more painstaking procedure than is usually employed, but it is essential for the highest possible accuracy.

Final Calculation of Percentages. With the intensities of the various element lines reduced to relative values compared to the internal standard iron lines, the next step is to reduce these ratios to percentage.

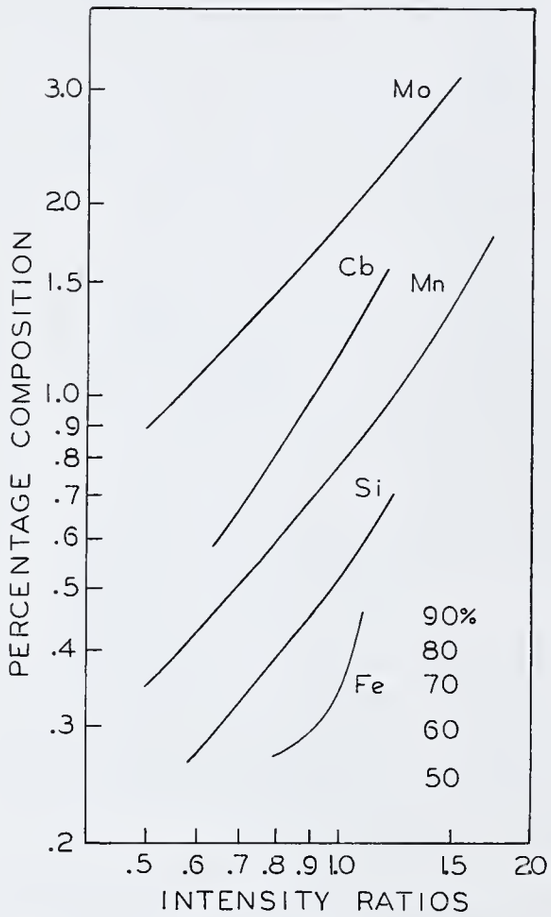

Figure 4. Working Curves for Molybdenum, Columbium, Manganese, and Silicon

With unity point correction curve for iron content of sample

The way to do this, so that a final working scale can be abstracted from the results, is to plot percentage as ordinates, and I_1/I_{Fe} (relative intensity to iron) as abscissa using log scales. This gives curves such as are shown in Figures 3 and 4. By replacing relative intensity values along the abscissa by the corresponding percentages, a rule for the direct reading of percentages is made available, to replace the log intensity scale of Figure 2. It should, of course, be adjustable horizontally. An arrangement like this is the basis for the conventional calculating machine.

So far this follows the usual procedure for the reduction of data to the final percentage. However, an innovation is introduced at this point to allow taking into consideration the variable iron content. Since the intensities of the iron lines will increase with the increased concentration of iron, a correction is made by moving the unity point of the relative intensities (Figures 3 and 4) to the right for an increase in iron. Thus, by introducing a curve for iron relating the percentage of iron to the intensity of the basic iron line, 2872 Å., and using this curve to locate the unity point of the system, a multicomponent system of analysis is made possible, instead of the essentially single-component system usually employed.

At first glance this appears involved, but actually it is simple. In most work, the approximate iron content is known. Thus, starting with this value, the unity point of the system is determined. The various elements are determined with respect to this iron value, and are then totaled, including the iron content. If they add up to 100 ± 2 per cent, the correct iron percentage was utilized. If the sum departs from 100 too much, the iron content must be evaluated in the following manner:

For the sum equal to a value over 100, the original iron estimate was too high. As a first trial, reduce the iron percentage by half the amount of the total exceeding 100 per cent. Recalculate the other elements and recheck the total. Readjust the iron value again if necessary, and recalculate.

In actual practice, the results are insensitive to variations in the iron percentage, especially at the higher iron percentages. Here an error in iron content of 5 per cent causes only a 2 per cent error in the element determinations. Thus, the iron content is usually known with sufficient accuracy just from the type of alloy being tested, so that a single calculation suffices for the determination of each of the other elements.

Comparison between Chemical and Spectrographic Determinations

Table IV indicates the degree of comparison to be expected between chemical and spectrographic results.

A number of chemical determinations were checked by two laboratories. The variations experienced were, in some cases, as great as those of the spectrographic method. Thus, in interpreting differences between the chemical results and the spectrographic results, a certain amount of the error must be attributed to the chemical method as well as the spectrographic. If the difference is shared between the two methods, the errors of the spectrographic method appear small enough for much routine work.

In the case of manganese, silicon, molybdenum, and columbium, the general agreement between the two methods was satisfactory. However, repeat chemical determinations on these elements, carried out by different laboratories, varied much more than repeat spectrographic results for these elements, so that no detailed comparison is given.

Table IV. Comparison of Methods

	Chromium		Nickel	
Sample No.	Chemical	Spectrographic	Chemical	Spectrographic
2001	16.5	16.7	10.2	9.9
2002	17.8	18.1	9.2	9.1
2003	18.6	18.2	9.2	9.0
2004	18.8	18.0	9.2	9.3
2005	19.1	19.5	10.6	10.5
2006	21.5	21.1	11.9	11.8
2007	13.3	13.2	...	...
2008	24.1	23.9	...	...
2009	17.4	17.7	12.5	12.4
2010	17.9	18.5	...	...
2011	20.0	19.8	10.0	10.5
2013	22.2	21.8	13.7	13.4
2014	26.0	25.7	...	...
2015	9.2	9.2	...	...
2016	23.4	23.4	20.2	20.2
2018	27.9	28.0	5.0	5.2
2021	11.9	11.4	...	...
2022	17.4	17.7	...	...
2023	18.4	19.0	8.6	8.2

Summary

This, then, represents a practical spectrographic method of stainless steel analysis. It is characterized by the ability to analyze any stock material of unknown metallurgical history in a simple and straightforward manner without elaborate sample preparation. It also introduces a means of using a variable constituent, iron, as an internal standard. These two advances make possible the determination of individual alloying elements in quantities over 20 per cent. This can be done even when the total alloying constituents amount to 45 per cent of the entire sample. The fact that steel stock of high percentage alloys of iron can be successfully analyzed by this method, suggests its use on the lower percentage alloy steels in cases where its sampling advantages are important.

Though the method is not so precise for the higher percentage alloying elements as chemical analyses carried out by ex-

perienced analysts working under carefully controlled conditions, it offers the advantages of a time-saving routine procedure for the analysis of many alloys produced by the steel industry.

Acknowledgment

The authors wish to express their appreciation to John Disario and A. P. Johnston, of Los Angeles, for their cooperation in certain phases of this problem.

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PRESENTED before the Third Annual Midwest Conference on Spectrographic Analysis, University of Michigan, Ann Arbor, Mich.

Acrolein Determination

In the Presence of Formaldehyde and Acetaldehyde by the Polarographic Method

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ACROLEIN can be identified by certain color tests (*2, 6, 7, 12*) and by the preparation of derivatives (*1, 8, 13*). The literature on its quantitative determination is meager.

Schiff's reagent has quantitative application (*9*). Neuberg (*10*) made use of the insolubility of the 2,4-dinitrophenylhydrazone for a quantitative precipitation method. Zappi and Labriola (*15*) studied the application of several aldehyde methods to the quantitative determination of acrolein, and found the Ripper bisulfite method satisfactory, as did Ivanov (*3*). Another method uses the reaction with iodine to give iodoform, with back-titration of the excess iodine (*5*).

The above methods are inaccurate when other aldehydes are present with acrolein. This paper describes the application of a reliable polarographic method to the quantitative determination of acrolein in the presence of formaldehyde and acetaldehyde.

Acrolein shows two reduction-potential waves (Figure 1). The first wave, presumably due to reduction of the double bond, has its half-wave potential at −0.83 volt at pH 4.8; −0.98 volt at pH 5.8; and −1.04 volts at pH 7.0 to 11.0, corrected for pool voltages. The second wave, due to reduction of the aldehyde group, has its half-wave potential at 1.44 volts at pH 8.7 to 11.0, corrected for pool voltage. The first acrolein wave at pH 7.5 appears at the flattened portion of the natural curve of the indifferent salt solutions. By use of the increment method (*11*) the beginning of this wave is easily observed, although the curve is somewhat steep at this point. The second acrolein wave at pH 7.5 is entirely obscured by the steepness of the natural curve in this potential range.

The fact that acrolein in alkaline solution produces two widely separated polarographic waves, offers two possibilities for determining it in the presence of other aldehydes often encountered in the same solution. The acetaldehyde wave is partially superimposed on the second acrolein wave, and therefore cannot be used for the determination of acrolein in the presence of acetaldehyde. The formaldehyde wave occurs between the two acrolein waves, and if the concentrations are not too great no overlapping occurs. Therefore either acrolein wave offers a possibility for its determination if formaldehyde is the only other aldehyde present. In a mixture of these three aldehydes acrolein can be determined from its first-appearing wave. At an elevated temperature and a lower pH the acrolein wave heights are greater. Reproducibility is better at a lower pH. A lithium phosphate-buffered solution at approximately pH 7 containing 0.01 molar lithium chloride proved to be the best indifferent salt solution for determining acrolein from its first wave.

Solutions

ALDEHYDE SOLUTIONS. The acrolein solution was prepared from Eastman Kodak Company acrolein by distilling it and collecting the 52° fraction in distilled water containing 0.01 per cent hydroquinone as stabilizer. The formaldehyde solution was made by diluting commercial formalin solution. The acetaldehyde solution was made from paraldehyde by distilling it in the presence of sulfuric acid and collecting the fraction, boiling point 22°, in distilled water containing 0.01 per cent hydroquinone. All aldehyde solutions were analyzed by the Ripper bisulfite method as modified by Kolthoff and Furman (*4*).

INDIFFERENT SALT SOLUTIONS, pH 11, were prepared by dissolving 1 gram of lithium carbonate and 0.01 mole of lithium chloride in water, adding 1 ml. of 0.2 per cent methyl red alcoholic solution and 1.5 ml. of 0.02 per cent bromocresol green alcoholic solution as maxima inhibitor, and diluting to 1 liter.

Solutions of pH 8.7 and 9.6 were prepared in the same way as the pH 11 solution and then carbon dioxide was added to adjust to the desired pH.

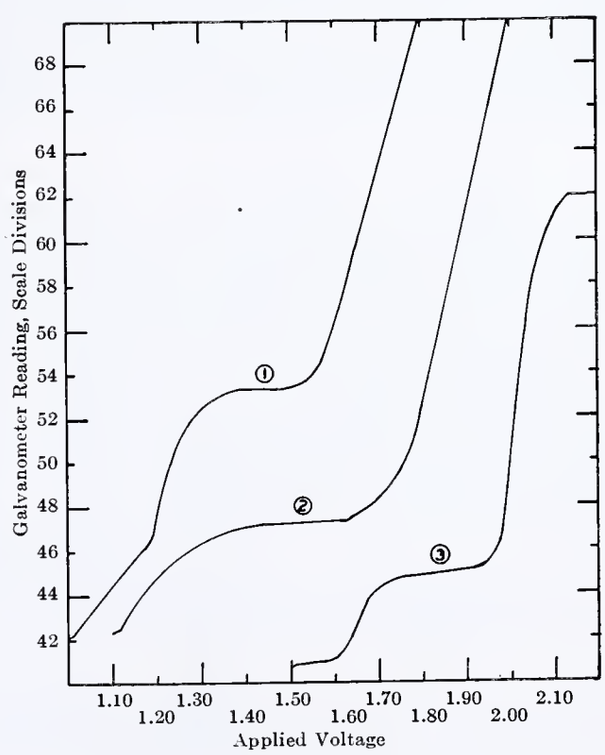

FIGURE 1. CURRENT VOLTAGE CURVES FOR ACROLEIN

Sensitivity, 1/5. Volume, 110 ml.
1. 2.71 mg. of acrolein, pH 7.5, 28.8°
2. Residual current, pH 7.5, 28.8°
3. 2.71 mg. of acrolein, pH 11.0, 24.8°

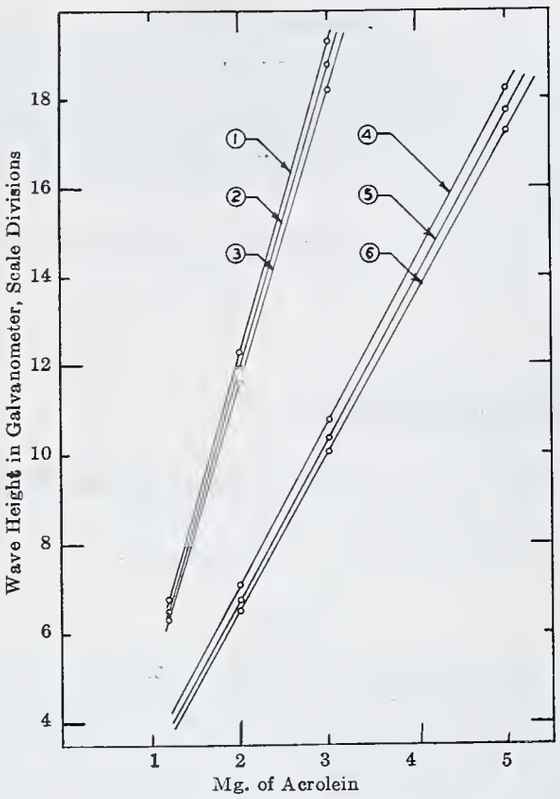

FIGURE 2. EFFECT OF TEMPERATURE ON WAVE HEIGHT

Sensitivity, 1/5. Volume, 110 ml.
Upper curves, acrolein second wave, pH 11
1. 28.8°. 2. 26.8°. 3. 24.8°
Lower curves, acrolein first wave, pH 7.5
4. 28.8°. 5. 26.8°. 6. 24.8°

The pH 7.5 solution was prepared by neutralizing 0.02 mole of phosphoric acid with lithium hydroxide, adding the maxima inhibitor, and diluting to 1 liter.

The pH 5.8 solution was prepared from potassium dihydrogen phosphate and sodium hydroxide (Clark and Lubs' standard mixtures) with maxima inhibitor added.

The pH 4.8 solution was prepared from potassium acid phthalate and sodium hydroxide (Clark and Lubs' standard mixtures) with maxima inhibitor added.

All solutions were measured for pH with a Leeds & Northrup glass electrode potentiometer electrometer.

Analyses

The polarograph used in this work was a visual-reading instrument known as the Elecdropode (manufactured by the Fisher Scientific Co., Pittsburgh, Penna.). All work was carried out at one fifth the maximum sensitivity of the galvanometer. The full sensitivity of the galvanometer is of the order of 0.006 microampere per scale division of 2.3 mm., the scale being 75 cm. (30 inches) from the galvanometer. Readings to one tenth of a scale division of the maximum swing of the galvanometer were made without difficulty.

The polarographic analyses were carried out in 125-ml. lipless beakers open to the air, since at lower sensitivities of the galvanometer the current due to the oxygen wave is not too great to interfere with aldehyde determinations (*14*). Further, the removal of oxygen with inert gas is time-consuming; and the suggested (*14*) use of bisulfite was found in the course of the present work to affect the wave heights of aldehydes. Since preliminary work gave erratic results which were found to be due in part to changes in temperature, a small constant-temperature bath was arranged to hold the cell during analysis.

The influence of temperatures and pH is well illustrated by the relative heights of the first acrolein waves (Figure 1, curves 1 and 3). Temperature-concentration curves were made (Figure 2). In the case of both acrolein waves, temperature control is necessary for accurate results since 1° of temperature change causes an 0.8 per cent error. The effect of pH on the accuracy of acrolein determinations may be seen in Figure 3, which shows curves for concentration-wave heights at pH 4.8 to 11 of the first acrolein wave. Curves for pH 4.8 and 11 are included, although the values are not reproducible, since when the analysis is repeated immediately on the same solution the wave height has become smaller. Figure 3 also shows the effect of pH on the second acrolein wave.

TABLE I. DETERMINATION OF ACROLEIN

Acrolein Present	Formaldehyde Present	Acetaldehyde Present	Wave Height[a] Found	Wave Height[a] Average	Acrolein Found[b]	Error	
Mg.	*Mg.*	*Mg.*			*Mg.*	*Mg.*	%
0.952	0.0	0.0	3.9 3.8	3.85	0.98	+0.028	+2.9
0.943[c]	17.7	6.14	3.5 3.6	3.55	0.93	−0.013	−1.3
1.904	0.0	0.0	6.0 6.1	6.05	1.83	−0.074	−3.9
1.904	10.6	6.14	6.2 6.1	6.15	1.87	−0.034	−1.8
2.856	0.0	0.0	8.9 8.6	8.75	2.90	+0.044	+1.5
2.856	0.0	6.14	8.9 8.7	8.80	2.91	+0.054	+1.9
3.808	0.0	0.0	11.0 11.0	11.0	3.78	−0.028	−0.7
3.808	17.7	0.0	10.9 11.1	11.0	3.78	−0.028	−0.7

[a] Galvanometer current increase in scale divisions as determined by increment method.
[b] See curve for pH 7.5 in Figure 3.
[c] Correction for concentration.

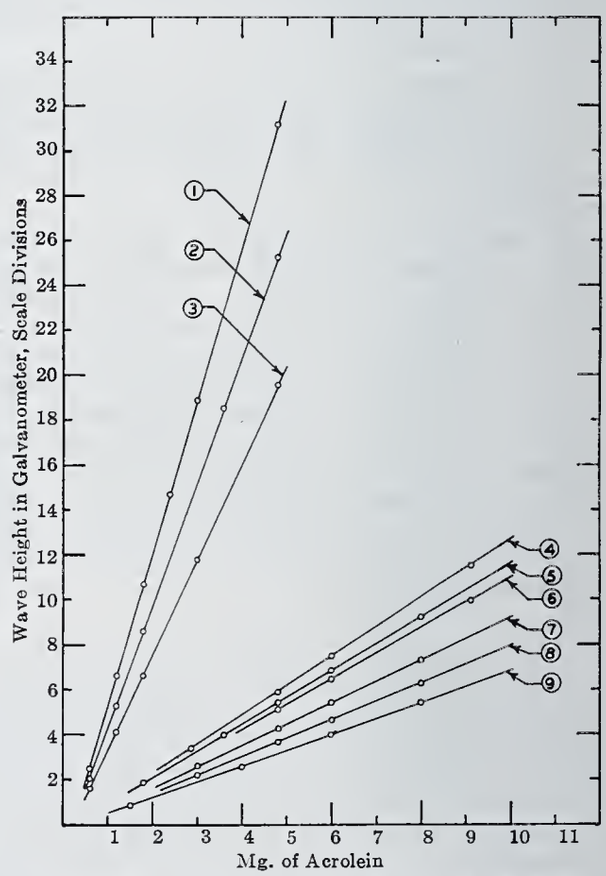

FIGURE 3. EFFECT OF pH ON WAVE HEIGHT

Sensitivity, 1/5. Temperature, 28.8°. Volume, 110 ml.

Acrolein second wave
1. pH 11.0
2. pH 9.6
3. pH 8.7

Acrolein first wave
4. pH 5.8
5. pH 7.5
6. pH 4.8
7. pH 8.7
8. pH 9.6
9. pH 11.0

Results

A buffered solution at pH 7.5 was selected as best for (1) reproducibility, (2) ease of observance of the beginning and end of the first acrolein waves by the increment method without the necessity of first plotting the polarographic curve, (3) maximum wave height, and (4) noninterference of formaldehyde and acetaldehyde. At the half-wave potential of −1.0 volt the drop weight was 0.0045 gram, and the drop rate was 4.02 seconds.

Table I contains representative data obtained at pH 7.5, 28.8° C., and a galvanometer sensitivity of one fifth.

Summary and Conclusions

The quantitative polarographic determination of acrolein in the presence of formaldehyde and acetaldehyde is most accurately made in a lithium chloride solution buffered at pH 7.0 to 8.0 with lithium phosphate. During the determination, the temperature must be held constant within ±0.05°.

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Woburn Iodine Absorption Method

Use in Combination with Partial Iodine Values for the Determination of Diene Numbers

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IN THE preceding paper (*22*) it was shown that iodine bromide solutions of 1.6 to 2 times the concentration of Hanus' solution can be used for determining the total unsaturation of oils and fatty acids containing conjugated double bonds. It was indicated that the "total iodine value" thus obtained could be used, in combination with partial iodine values, for determining the diene value of certain oils and fatty acids.

Previous Methods

The use of total and partial iodine absorption values for determining conjugation is not new.

Kaufmann (*14*) measured the iodine values of eleostearic acid for saturation of one, two, and three double bonds, using bromine with and without irradiation and thiocyanogen, respectively, in an attempt to calculate the composition of tung oil. Qualitative methods for showing the presence of conjugated double bond systems based on their characteristic rapid primary absorption of Wijs' reagent which is followed by a slow completion of the addition, were used by Boeseken (*3*, *4*), Gelber (*10*), and later by Forbes and Neville (*9*), while Kass *et al.* demonstrated that the conjugation could be qualitatively detected by the use of a rapid Hanus method in conjunction with the Rosenmund-Kuhnhenn and other modified Wijs procedures (*11*). Scheiber (*27*) and Boettcher (*5*) used Hanus' solution for the same purpose, while Smit (*28*) made use of a similar behavior toward oxidation with peracetic acid.

Toms (*30*) calculated the percentage of eleostearic acid in tung oil from the difference between the bromine-vapor iodine value (*26*) and the Wijs iodine value, and his method was later improved by Bolton and Williams (*6*), who replaced the ordinary Wijs value by the "instantaneous" iodine value, found by extrapolating two Wijs iodine numbers for 0.5 and 3 hours, respectively.

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The need for such differential halogen absorption methods was temporarily placed in doubt when in 1936 Kaufmann and Baltes (*15*) and subsequently Ellis and Jones (*8*) completed direct methods for determining the diene value, using maleic anhydride according to the Diels-Alder reaction. While a quantitative application of differential halogen absorption had been made only in the case of tung oil, the maleic anhydride addition methods were equally applicable to systems of two and three conjugated double bonds. However, difficulties arose in the presence of hydroxyl groups, especially with the more rapid Ellis-Jones method (*7*, *25*) and attempts to overcome these by acetylation were only partially successful (*1*). The Kaufmann-Baltes method is said to be unaffected by hydroxyl groups, and tests made in this laboratory showed the hydroxyl group of hydrogenated castor oil (*25*) to cause no disturbance with this procedure. Bickford and collaborators (*1*), however, obtained diene values on castor oil of 3.2 with the Kaufmann-Baltes method, and of 9.9 to 10.2 with the Ellis-Jones method. Bruce and Denley (*7*) found a diene value of 19.7 for commercial castor oil with the Ellis-Jones method.

An imperfection in dienometry was disclosed when McKinney and Jamieson (*18*) were unable to obtain theoretical maleic anhydride addition with alpha- and beta-eleostearic acid and the alpha-triglyceride. These findings were largely confirmed by Norris, Kass, and Burr (*24*), who reported the addition of eleostearic acid to be only in the neighborhood of 85 per cent of the theoretical. Certain isomers of eleostearic acid are said to be unable to add maleic anhydride although they contain the conjugated structure (*13*).

Such observations have not diminished the usefulness of the maleic anhydride addition methods, which are frequently

employed in industrial analysis. It is evident, however, notwithstanding the increasing use which is being made of spectroscopic methods for determining conjugation, that an independent method based on differential halogen absorption is desirable.

Quantitative Relationship

When Toms (*30*) in 1928 calculated the percentage of alpha-eleostearic triglyceride in tung oil from the formula $(q - p)\frac{100}{87.7}$, where q and p are the total and partial iodine values, he unwittingly introduced the concept of diene value, although this term was suggested only 8 years later by Kaufmann and Baltes (*15*). The number 87.7, which he calculated as the difference between the iodine values for the addition of 18 and 12 atoms of iodine, respectively, to the triglyceride is actually the diene value according to the later definition, which was based on the consideration, that for each molecule of maleic anhydride added one double bond less than originally present remains. The relationship, total iodine value minus partial iodine value equals diene value, is a general one, if partial iodine value is understood to represent the value obtained when all nonconjugated double bonds, one half of all conjugated double bonds occurring in pairs, and two-thirds of all conjugated double bonds occurring in triplets, have added iodine. That this is necessarily so may be shown as follows:

Let a mixture contain x per cent of nonconjugated substances with an iodine value of X, y per cent of substances having a pair of conjugated double bonds with a true or total iodine value of Y, and z per cent of substances having three conjugated double bonds with a total iodine value of Z. Each of the three portions may in itself be a mixture of various constituents, in which case X, Y, and Z represent the average iodine values of each portion. The total iodine value of the mixture is then

$$I.\ V._T = \frac{1}{100}(xX + yY + zZ)$$

its partial iodine value by the above definition:

$$I.\ V._P = \frac{1}{100}(xX + {}^1/_2yY + {}^2/_3zZ)$$

and its diene value:

$$D.\ V. = \frac{1}{100}({}^1/_2yY + {}^1/_3zZ)$$

The difference, total minus partial iodine value, is then:

$$\frac{1}{100}(xX - xX + yY - {}^1/_2yY + zZ - {}^2/_3zZ) = \frac{1}{100}({}^1/_2yY + {}^1/_3zZ)$$

which equals the expression for the diene value.

Application to Dienometry

In order to use this relationship for the determination of the diene value of a substance it is necessary to select adequate methods for the determination of the two iodine values, total and partial.

Total Iodine Value. There exists, to the authors' knowledge, no halogen absorption method that can claim the accuracy and universality of properly conducted quantitative hydrogenation procedures. However, these require time-consuming preparations and a complicated apparatus which is not ordinarily available in the average industrial laboratory.

Table I. Partial Iodine Values of 9,11- and 10,12-Octadecadienoic Acids

Sample Weight	Wijs Solution	$CHCl_3$	Time	Excess	I. V. Found	Partial I. V., Theoretical	Material
Gram	*Ml.*	*Ml.*	*Min.*	%			
0.1218	12	10	2	163	92.2	90.5	9,11-acid
0.1887	20	10	2	173	95.2	90.5	9,11-acid
0.3232	32	10	2	159	93.8	90.5	9,11-acid
0.1832	24	10	0.5	239	94.7	90.5	9,11-acid
0.2994	20	20	2	80	91.5	90.5	9,11-acid
0.2856	16	16	2	52	90.8	90.5	9,11-acid
0.1396	8	8	2	60	89.9	90.5	10,12-acid
0.2023	11	11	2	52	89.8	90.5	10,12-acid

The Woburn iodine absorption method (*22*), on the other hand, has all the required simplicity and speed at only a slight sacrifice in accuracy. It fulfills the necessary prerequisite of measuring the total unsaturation for both conjugated and nonconjugated substances, which is not possible with the conventional absorption methods.

The difficulty in its application to substances of mixed unsaturation lies in the fact that threefold conjugation, as in eleostearic acid, is best measured with the 0.40 N solution at 0° C., while 9,11-linoleic acid gives somewhat high results under these conditions and is better characterized by 0.32 N iodine bromide solution under otherwise similar conditions. If the 0.32 N solution was used for 1 hour at 20° C., however (method A), fair agreement with theoretical values was obtained in either case, and this modification, therefore, shows promise for total iodine value determination when systems with both two and three conjugated double bonds are present. If either double or triple conjugated systems are known to predominate, the accuracy may be improved by modifying the conditions slightly, according to the findings of the previous investigation.

Partial Iodine Value. This value in the past, has received attention only in the case of tung oil (*12*). The ordinary Wijs iodine value was considered to represent this stage adequately, where two out of three double bonds in eleostearic acid had been saturated with halogen, until Bolton and Williams (*6*) extrapolated a time series of Wijs numbers of tung oil and obtained its true partial or instantaneous iodine value. A simpler procedure, requiring one 2-minute determination in an ice bath instead of two exposures of 0.5 and 3 hours, respectively, was suggested by one of the authors (*21*) and shown to give the theoretical two-thirds addition for alpha-eleostearic acid. If this method is to be used in the determination of the diene value it must be adapted to systems of two conjugated double bonds as well.

The best-known example of a fatty acid containing a system of two conjugated double bonds is Mangold's 9,11-linoleic acid, formed by the dry distillation of ricin–elaidic acid (*19*). After repeated recrystallizations from petroleum ether, samples of this acid (m. p. 53. 5–54° C.) were subjected to the modified Wijs method in ice, as previously suggested for tung oil (*21*). The values obtained (Table I) are close to the theoretical partial iodine value, but from 2 to 5 points higher. It is evident that the addition of halogen to the second double bond proceeds easier than the corresponding addition to the third double bond in eleostearic acid.

In order to provide for somewhat milder conditions, a smaller excess of Wijs solution was employed, and instead of using 10 ml. of chloroform as solvent for the sample with 25 ml. of Wijs solution, an equal volume of chloroform and Wijs solution was employed. The resulting values agreed with theory within one unit (Table I, lines 5–6).

A new solid conjugated isomer of linoleic acid was found in the fatty acids resulting when dehydrated castor oil (Isoline, unbodied) was isomerized, using a commercial process for shifting isolated double bonds into the conjugated position (*31*). Disruptive oxidation showed that this new acid, melting at 57° C., which is not identical with Mangold's acid, is 10,12-octadecadienoic acid (*20*).

Table II. Iodine Numbers

Fatty Acid	Wijs Iodine No. (0.5 Hr.)	Woburn Iodine No. Method A (0.32 *N*)	Woburn Iodine No. Method D (0.4 *N*)	Partial Iodine No.	% Excess Short Wijs Iodine No.	Woburn Diene No.
Linseed, split	189.0	185.7	189.5[a]	190.6	268	−4.9 or −1.1
Sunflower, distilled	132.0	133.5	....	132.0	237	1.5
Soybean, split	146.5	143.7	....	145.7	229	−2.0

[a] Method D was used on linseed fatty acids because lower iodine values were found with method A.

After repeated recrystallizations from petroleum ether, ethyl ether, and ethyl alcohol, the new acid, which was handled in an atmosphere of carbon dioxide to prevent oxidation, gave almost theoretical partial iodine values when analyzed according to the procedure found most suitable for Mangold's acid (Table I, lines 7–8).

In analyzing mixtures of fatty acids containing both types of conjugation—i. e., double and triple—it must be borne in mind that the conditions giving the most accurate results are not identical in the two cases. That it is possible, however, to obtain almost theoretical partial iodine values with blends containing both types of conjugation is seen by comparing Tables III and IV. In determining the partial iodine value of such blends it is preferable to keep the excess of reagent between 200 and 250 per cent and follow the procedure used with tung oil. This is necessary in the presence of linolenic acid, which otherwise is not completely saturated, owing to the somewhat retarded addition of halogen to this nonconjugated acid.

Previously, it had been thought that the partial iodine number gave low results with nonconjugated oils and fatty acids, particularly those containing linolenic acid, because not enough time elapsed for the halogen to add completely to the last isolated double bond. However, the iodine values given in Table II for some fatty acids indicate that the partial iodine method may be used to determine the total unsaturation of nonconjugated fatty acids, including linseed fatty acids, if the excess of the reagent is 200 to 250 per cent. It may also serve as a method of control in isomerizing fatty acids to their conjugated form.

The determination of total iodine values was as follows:

Method A, 0.32 *N* IBr, 1 hour at 20° C. Method B, 0.40 *N* IBr, 3 hours at 0° C. Method C, 0.32 *N* IBr, 3 hours at 0° C. Method D, 0.40 *N* IBr, 1 hour at 20° C. The partial iodine values were determined in thin-walled Erlenmeyer flasks in the ice bath as previously described (*21*), following the details originally suggested for tung oil—i. e., 200 to 250 per cent excess of Wijs solution (25 ml.), 10 ml. of chloroform, and 2 minutes, unless otherwise stated.

Diene Values. These were determined by subtracting partial iodine values from the total (Woburn) values and compared to theoretical figures and, in some cases, to conventional (maleic anhydride) diene determinations (Table III).

The blends analyzed (Tables III and IV) were made up as follows:

Blend A. 50.0% 9,11-octadecadienoic acid
50% soybean fatty acids (I. V. = 138.8)

Blend B. 5.505% beta-eleostearic acid
31.40% 10,12-octadecadienoic acid
63.095% sunflower fatty acids (I. V. = 132.0)

Blend C. 35.71% beta-eleostearic acid
34.25% 10,12-octadecadienoic acid
30.04% linseed fatty acids (I. V. = 179.3)

The diene values obtained for beta-eleostearic acid were 89.7 and 90.8, depending on details followed in the determination of the total unsaturation. The latter value, which is based on method B, agrees well with the calculated value. This method, which had previously been recommended for tung and oiticica oils (*22*, page 788) was employed for calculating the diene value of two of the tung oil samples previously described (*22*, page 786). The resulting values agree well with the corresponding Ellis-Jones maleic anhydride diene values. The iodine value of 224.2 for sample 4, which coincides with the break in the curve for varying excess (*22*, page 787), gives a diene value somewhat lower than those by either of the conventional methods.

Table III. Calculated Values of Blends

Blend	Total Iodine Value	Partial Iodine Value	Diene Value
A	160.0	114.7	45.3
B	155.2	121.7	33.4
C	213.5	150.0	63.6

With oiticica oil the use of the ice bath (method B) is especially necessary because of the known influence of the keto group in licanic acid on iodine value determinations at ordinary temperatures. While conventional iodine absorption procedures fail entirely with this oil (*11*), both the total and partial iodine values determined with the methods here described gave reproducible results and the differential diene value lay between the Ellis-Jones and the Kaufmann-Baltes values.

While 9,11-linoleic acid gave satisfactory results, the differential diene value of the new 10,12-linoleic acid was several points too high. The methyl ester of this acid, however, gave practically theoretical diene values. Blend B, which contained the new acid as the principal conjugated constituent, also gave a somewhat high differential diene

Table IV. Comparison of Differential Iodine Absorption and Diene Values

Substance	Woburn Iodine Value	Partial Iodine Value	Diene Value by Difference	Maleic Anhydride Diene Value	Theoretical Diene Value
Beta-eleostearic acid	272.7 (A) 273.8 (B)	183.0	89.7 90.8	.....	91.2
Tung oil 4[a]	226.6 (B) 224.2[b]	160.5	66.1 63.7	75.0 (KB) 66.2 (EJ)	.. ..
Tung oil 5[a]	227.1 (B)	159.5	67.6	73.5 (KB) 69.1 (EJ)	..
Oiticica oil, sample 1	203.9 (B)	139.7	64.2	71.4 (KB) 59.5 (EJ)	..
Oiticica oil, sample 2	201.6 (B)	138.3	63.3	60.3 (EJ)	..
9,11-Linoleic acid	183.3 (A)	91.2[c]	92.1	.....	90.5
10,12 - Linoleic acid	186.7 (A)	89.9[c]	96.8	.....	90.5
10,12-Methyl linoleate	173.4 (A)	86.4[c]	87.0	.....	86.2
Blend A	160.6 (A)	113.3[c]	47.3	.....	45.3
Blend B	161.8 (A) 157.3[d]	124.1 ..	37.7 33.2	....	33.4 ..
Blend C	213.0 (A)	149.1	63.9	.....	63.6
Castor fatty acid	89.3 (A)	87.0	2.3	.....	..
Walnut oil	157.4 (A)	156.1	1.3	.....	..

[a] Samples described in (*22*).
[b] From excess *vs.* iodine value curve in previous paper (*22*).
[c] 50 to 80% excess reagent; volumes of Wijs solution and chloroform equal.
[d] 390% excess of reagent.

Table V. Comparison of Diene Values for Commercial Products and Samples

Substance	Woburn Iodine Value[a]	Partial Iodine Value	Diene Value by Difference	Maleic Anhydride Diene Value
Conjulin F. A.[b], sample 1	190.4 (D)	117.5	72.9	18.0 (EJ)
Conjulin F. A., sample 2	178.7 (D)	116.2	62.5	24.7 (EJ)
Conjulin F. A., sample 3, distilled	186.0 (D)	131.9	54.1	.....
Conjusoy F. A.[b]	136.7	94.4	42.3	14.1 (EJ)
Conjusoy F. A., distilled	137.1	95.4	41.7	8.8 (KB) 10.5 (EJ)
Dehydrated castor oil, sample 1	155.1	122.2	32.9	18.4 (KB)
Debydrated castor oil, sample 2	155.3	118.2	37.1	13.5 (KB)
Dehydrated castor fatty acid, distilled	163.9	130.0	33.9	22.8 (EJ)
Isomerized isoline[c] fatty acids	165.4	101.2	64.2	39.2 (EJ)
Isomerized walnut fatty acids	159.6	96.5	63.1	.....
Heat-treated oiticica oil[d]	182.4 (B)	121.9	60.5	42.1 (EJ)

[a] Method A, except when otherwise indicated.
[b] Conjulin F. A. and Conjusoy F. A. are trade names for isomerized linseed and soybean fatty acids, respectively.
[c] Isoline is tbe trade name for dehydrated castor oil as manufactured by Woburn Degreasing Company of N. J.
[d] This sample, known in the trade as Cicoil, was obtained through the courtesy of the Brazil Oiticica Company. Its viscosity was 21 poises.

value, except when a smaller excess of reagent than usual was employed in the Woburn iodine value determination.

Summing up the data presented in Table IV, in most cases the observed values are in good agreement with theory. The diene value determination by differential halogen absorption, in its present form, may therefore be accepted as a simple and rapid method for obtaining a good quantitative estimate of conjugation in fatty acids and esters. Certain limitations in the scope of applicability are discussed below.

Limitations

There are certain compounds, other than those containing conjugated double bonds, which behave abnormally with halogenating reagents. They, too, add variable amounts of halogen, depending on the length of contact, excess of reagents, kind of reagent used, and other secondary factors.

Among such compounds van Loon (*17*) lists hydroxy acids, oils with much unsaponifiable matter, polymerized oils or acids, and 6,7-stearolic acid containing an acetylenic or triple bond. With polymerized oils and esters, this behavior was also observed by Steger and van Loon (*29*) and by Kino (*16*) who traced the increased iodine value of these products upon prolonged time of contact to substitution. Morrell (*23*) found that the iodine values of the maleic anhydride–licanic acid addition products were too high after long treatment with Wijs reagent and ascribed it to keto-enolization. Rossmann (*26*) tested undecylenic and cinnamic acids among compounds with sterically hindered or inactive bonds requiring larger excess of bromine vapor for complete saturation.

It might be thought that any of these compounds would give a higher iodine value with the Woburn method than with the modified Wijs procedure and, therefore, show an apparent diene value. However, it was shown in the case of castor fatty acids (Table IV) that the difference between the two iodine values was only 2.3 points. Even if it is assumed that this was caused entirely by the hydroxyl group of ricinoleic acid and that no conjugated acid was present, the error caused by the hydroxyl group is small. The resulting differential diene value is smaller than those observed on castor oil with either of the methods using maleic anhydride (*1*, *7*). The values for oiticica oil (Table IV) similarly show that the keto group does not affect the differential halogen absorption values adversely if the Woburn iodine value is determined at 0° C. (method B).

On the other hand, the authors' observations indicate that polymerized oils definitely have differential iodine absorption values, regardless of the presence of conjugated double bonds. Their method will, therefore, not be applicable to bodied oils. This may be due not only to substitution, as observed by Kino (*16*), but to sterically hindered double bonds remaining in cyclic polymers as well. Other types of inactive bonds might also result in an apparent diene value and this factor must be considered whenever substances of unknown or doubtful composition are tested by the new method.

Listed in Table V are the constants of a number of substances for which the Woburn diene value differs markedly from the diene values obtained by other methods, the difference being definitely larger than any experimental errors previously observed. The isomerized fatty acids listed in this table have been made semicommercially by treatment with aqueous alkali (patents pending), designed to cause the shifting of double bonds from the isolated to conjugated position (*31*). The two dehydrated castor oils were unbodied samples having viscosities of F-G (Isoline, raw) and G-H in the order listed.

In all these oils or fatty acids the diene value by difference is considerably larger than the conventional maleic anhydride values. The question naturally arises whether this disagreement is due to the presence of some of the above discussed inactive double bonds in the isomerized products, to a failure of maleic anhydride to react with all conjugated double bonds present, or to both these causes. To decide this, tests were made to determine whether the extent of maleic anhydride addition could be increased.

Table VI. Diene Values with Maleic Anhydride in Orthodichlorobenzene

Substance	Period of Refluxing	Diene Values
	Hours	
Tung oil, sample 5[a]	3	69.1, 69.7
Conjulin F. A.[b]	3	50.4, 50.8
Conjusoy F. A.[b]	1	26.8
	2	35.4
	3	40.6, 41.9
	18	44.7, 46.0
Soybean fatty acid distilled	1	3.8
	2	14.7
	3	11.9, 16.9
	18	47.6

[a] Sample described in (*22*).
[b] Trade names.

Maleic Anhydride at Higher Temperatures

The use of higher boiling solvents in the refluxing operation of the Ellis-Jones method suggests itself as a means of promoting the addition. Of several solvents which were tried for this purpose, *o*-dichlorobenzene (technical), boiling at 175° C., appeared most promising. The details of these determinations were similar to those of the Ellis-Jones method, except that enough ether was added before hydrolyzing to obtain a mixture floating on water, thus facilitating the extraction of residual maleic anhydride in the separatory funnel. Blank runs were made as usual under identical conditions. They showed that no appreciable reaction took

place between maleic anhydride and the solvent during the refluxing period.

Some diene values thus obtained are listed in Table VI. The values for tung oil are seen to be identical with those obtained in toluene solution in the Ellis-Jones procedure (compare Table IV). All values obtained on conjugated soybean and linseed fatty acids, on the other hand, were much higher than the figures obtained with the Ellis-Jones or the Kaufmann-Baltes methods (compare Table V). With Conjusoy fatty acids the 3-hour values equaled the differential iodine absorption values, but with Conjulin fatty acids they were somewhat lower.

Quantitative conclusions which might be drawn from these maleic anhydride diene values at higher temperature are made difficult, however, by the fact that ordinary distilled soybean fatty acid, when similarly treated in *o*-dichlorobenzene solution, also led to substantial diene values. The values obtained, with both conjugated and nonconjugated fatty acids, depended on the length of refluxing, suggesting that a rearrangement of the double bonds into the conjugated position takes place during the determination. [The reaction of maleic anhydride with nonconjugated fatty acid esters at 200° to 250° C. has been studied thoroughly by Bickford *et al.* (*2*).]

For short refluxing periods the addition values of the isomerized fatty acids are much larger than those of the unisomerized, distilled fatty acids, and the difference between the two corresponding values is larger than the conventional diene values for the conjugated fatty acids. It follows, therefore, that the amount of conjugation originally present is larger than would be indicated by the conventional diene value. The 1-hour values listed show, for example, that the true diene value of the Conjusoy sample is certainly not below 23—i. e., 26.8 − 3.8—although it may be higher. The value of 14, obtained with the Ellis-Jones method on the same sample (Table V) was, therefore, due to incomplete addition. Similar conclusions must be drawn concerning the Kaufmann-Baltes method, since both methods gave comparable results with the conjugated products.

Further evidence that the conventional methods yield incomplete diene values with these products is seen in a comparison with spectroscopic data. (The spectroscopic determinations are taken from a private communication by Bradley and Richardson, whose courtesy in permitting its use is gratefully acknowledged.) The spectroscopically calculated diene values of the samples of Conjulin and Conjusoy fatty acids, listed in Table V, were 36.9 and 29.8, respectively. Assuming these values to be correct, the conventional diene values would indicate only about one half of the conjugated double bonds present in these products. The Woburn diene values, on the other hand, would be too high.

Summary

The quantitative relationship, total iodine value − partial iodine value = diene value, can form the basis for determining the diene value of fatty acids and oils.

The Woburn iodine method, which makes use of iodine bromide solutions 1.6 to 2 times the concentration of Hanus' solution, may serve to determine total iodine values.

The partial iodine values may be determined with Wijs solution, by limiting the time of contact to 2 minutes at ice-bath temperature. This procedure is almost similar to one previously evolved for tung oil. Theoretical partial iodine values are obtained for Mangold acid and for a new solid conjugated isomer of linoleic acid—i. e., 10,12-octadecadienoic acid—showing that this method is applicable to conjugated dienes. The modified Wijs procedure can be used to determine total unsaturation in nonconjugated fatty acids, by using the correct excess of reagent.

The diene values obtained by differential iodine absorption for tung oil and oiticica oil compare well with maleic anhydride diene values. The total and partial iodine values of oiticica oil are reproducible in spite of the inapplicability of other iodine absorption procedures to this oil. With pure conjugated fatty acids and esters, the differential diene values, in general, are in good agreement with calculated values.

The new method is not applicable in the presence of polymerized oil and certain types of unsaturation, which may cause differential iodine absorption due to steric hindrance.

Certain commercial fatty acids and oils of the conjugated type have differential diene values much higher than their maleic anhydride addition values, determined by standard methods.

Maleic anhydride does not react completely with the conjugated double bonds present in these manufactured products at the temperature used in the standard diene methods.

A slow reaction takes place between maleic anhydride and nonconjugated fatty acid in *o*-dichlorobenzene and the amount of anhydride added after 18 hours equals that added to the corresponding conjugated fatty acid.

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Analytical Data for the Systems Carbon Tetrachloride–Acetic Acid–Benzene and Carbon Tetrachloride–Tetrachloroethylene

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IN THE course of investigations on the ternary system benzene–carbon tetrachloride–acetic acid and the binary system carbon tetrachloride–tetrachloroethylene, it was found necessary to have methods of analysis for each system. Two methods were developed for the ternary system and the analytical data obtained for the binary system.

The carbon tetrachloride, benzene, and tetrachloroethylene were of the best grade obtainable from the Eastman Kodak Company. The acetic acid used was Baker's Analyzed material. As a check on purity, the carbon tetrachloride and tetrachloroethylene were redistilled. The boiling range of the carbon tetrachloride was found to be 0.3° C. and that of the tetrachloroethylene, 2° C. The middle portion of the latter compound was used. The refractive indexes of the solvents are given in Table I.

TABLE I. REFRACTIVE INDEXES

	Experimental	Literature (*2*)
Benzene	1.4985 at 25° C.	1.5017 at 20° C.
Carbon tetrachloride	1.4549 at 25° C.	1.4630 at 20° C.
Acetic acid	1.3706 at 25° C.	1.3715 at 22.9° C.
Tetrachloroethylene	1.4993 at 25° C.	1.5055 at 20° C.

Procedure and Results

In the first method of analysis for the ternary system, the sample was weighed, and the acid determined volumetrically with standardized sodium hydroxide solution. Thymol blue was used as the indicator. Phenolphthalein as ordinarily prepared in alcoholic solution could not be used because the alcohol changes the refractive index of the benzene–carbon tetrachloride layer. However, thymol blue made up in a basic aqueous solution (*1*) proved satisfactory. The layer or phase containing benzene and carbon tetrachloride appears as the titration is carried out. The other layer consists of water, sodium acetate, thymol blue, and excess sodium hydroxide. The two layers were separated in a centrifuge, and the refractive index of the benzene–carbon tetrachloride layer was determined in an Abbe refractometer, which was held at 25.0° ± 0.1° C. The index of refraction was then compared with a standard curve (Figure 1) that represented the refractive indexes of synthetic mixtures of benzene and carbon tetrachloride plotted against the per cent by weight. These synthetic mixtures were made up by volume at the 10 per cent divisions. Corrections were made for the densities of the liquids at room temperature. The values for the refractive indexes at 25° are given in Table II.

That the method of analysis is capable of sufficient precision to be useful was demonstrated by the following experiments.

Five mixtures of carbon tetrachloride and benzene covering the whole range of the solution (refractive indexes 1.4642, 1.4750, 1.4816, 1.4918, and 1.4963) were made up and (*a*) the refractive indexes of the solutions were determined. Then equal quantities of the solutions were mixed with (*b*) water, (*c*) water and sodium acetate solution, (*d*) water and sodium hydroxide, and finally (*e*) indicator solution. The last four mixtures, in each case, were shaken thoroughly and then allowed to stand for 0.5 hour. Finally they were centrifuged for about 2 minutes and the refractive index of the carbon tetrachloride–benzene layer was redetermined. It was found that the values had not changed by more than ±0.0005 in any sample.

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TABLE II. BENZENE AND CARBON TETRACHLORIDE

Benzene, Weight %	Refractive Index at 25° C.
0	1.4549
10	1.4626
20	1.4693
30	1.4744
40	1.4793
50	1.4837
60	1.4873
70	1.4906
80	1.4936
90	1.4961
100	1.4985

The results from the sample having refractive index 1.4750 are given below as representative of the group:

Sample	Refractive Index
(*a*)	1.4750
(*b*)	1.4749
(*c*)	1.4749
(*d*)	1.4751
(*e*)	1.4750

It may be concluded that solubility effects are not great enough to change seriously the refractive index of mixtures of benzene and carbon tetrachloride.

A preliminary run on the analysis of the mixture was performed. Three milliliters of acetic acid in water required 77.42 ml. of sodium hydroxide solution (27 grams of sodium hydroxide per liter) for neutralization; 1 ml. of acetic acid in 6 ml. of the sample of carbon tetrachloride and benzene whose refractive index is given above, required 26.00 ml. of sodium hydroxide solution for neutralization. This is a check within 1 per cent. The layer of carbon tetrachloride and benzene was separated and found to have a refractive index of 1.4754, which is in good agreement with the previous values. Three solutions containing benzene, carbon tetrachloride, and acetic acid were then made up

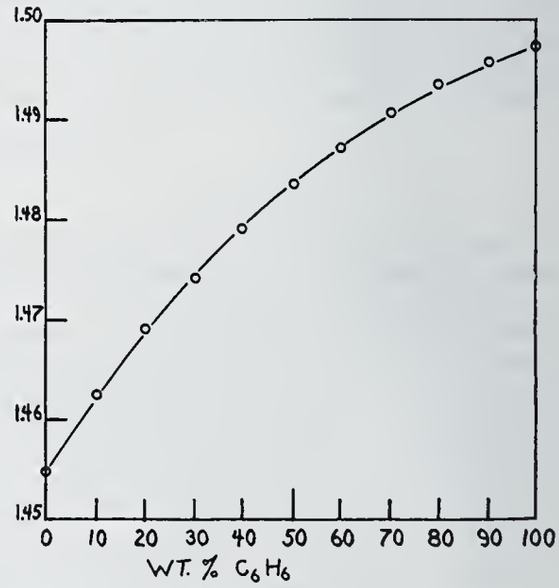

FIGURE 1. REFRACTIVE INDEX OF SYSTEM BENZENE–CARBON TETRACHLORIDE AT 25° C.

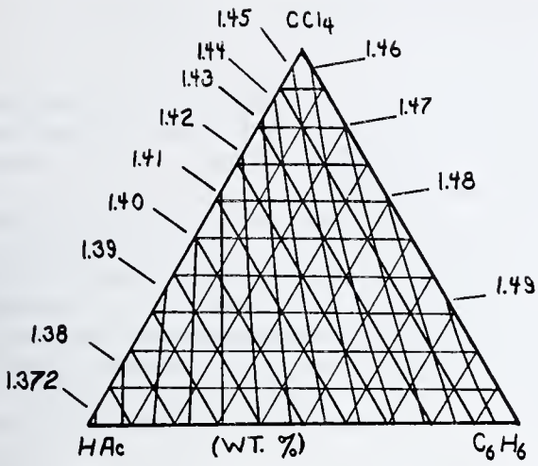

Figure 2. Lines of Constant Refractive Index for Ternary System at 25° C.

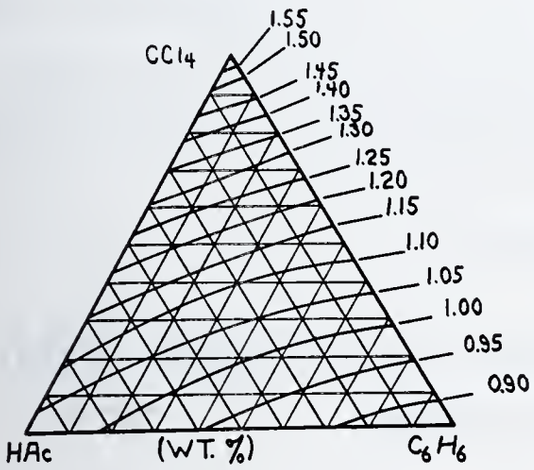

Figure 3. Lines of Constant Density for Ternary System

accurately at approximately the following concentrations, expressed in per cent by weight:

Benzene	Carbon Tetrachloride	Acetic Acid
10	10	80
80	10	10
10	80	10

The corresponding percentages, as determined by the method of analysis outlined above, were in all cases within 1 per cent.

A more rigorous experiment was then performed. Twelve solutions were made up, each with 5 ml. of water, 1 ml. of acetic acid, and 3 drops of thymol blue solution. Solutions 3, 4, 11, and 12 each had 1 ml. of carbon tetrachloride. Solutions 5 and 6 each had 5 ml. of carbon tetrachloride. Solutions 7, 8, 11, and 12 each had 1 ml. of benzene. Titrations were carried out for the acid with standardized sodium hydroxide solution:

Solution	Titer
1	29.75
2	29.88
3	29.78
4	29.85
5	29.77
6	29.87
7	29.70
8	29.65
9	29.91
10	29.92
11	29.72
12	29.85
	Av. 29.80

It is felt that these experiments demonstrate satisfactorily that the method of analysis is practicable and that mixtures containing other water-soluble acids might be analyzed in a similar way.

In later work the analysis of the ternary system was based on the measurement of the density and refractive index.

Representative mixtures were made up covering the three-component diagram and their refractive indexes were determined at 25.0° ± 0.1° C. The densities were determined by the "loss of weight principle". A drop of Pyrex brand glass was suspended in the liquids by means of a fine tungsten wire. Rigid temperature control over the mixtures was not necessary because the change of density with change of composition was so great. The densities were measured at 27° ± 0.5° C. Sixteen and fifteen samples were used to construct the ternary density and refractive index diagrams, respectively. In transposing the refractive index data to the ternary diagram, six graphs were made, one along each 0 per cent line and one along each 25 per cent line (total of 6), plotting refractive index against per cent by weight. It was then decided what intervals would be useful to plot. For refractive index, the 0.01 intervals were marked on the six graphs—that is, 1.40, 1.41, 1.42, etc. These values were then marked on the ternary diagram at the appropriate compositions. A line was drawn through all points representing the same value of refractive index (Figure 2). The same procedure was followed in transposing the experimental density data to the ternary graph (Figure 3).

The experimental refractive index and density data are presented in Tables III and IV, respectively.

The analysis of the binary mixture, carbon tetrachloride–tetrachloroethylene, was most conveniently done by refractive index. Solutions were made up accurately by weight at

Table III. Refractive Indexes of Ternary Mixtures

Acetic Acid, Weight %	Benzene, Weight % 0	25	50	75	100
100	1.3706	....	....	....	....
75	1.3851	1.4011	....	....	....
50	1.3991	1.4184	1.4330	....	....
25	1.4229	1.4425	1.4555	1.4657	....
0	1.4549	1.4724	1.4837	1.4923	1.4985

Table IV. Densities of Ternary Mixtures

Acetic Acid, Weight %	Benzene, Weight % 0	20	25	50	70	75	80	100
100	1.0304	....	....	....	....	....	....	....
75	1.1287	....	0.9846	....	....	....	....	....
50	1.2410	....	1.0603	0.9314	....	....	....	....
25	1.3825	....	1.1675	1.0102	....	0.8966	....	....
0	1.5866	1.3533	....	1.1234	1.0091	....	0.9507	0.8734

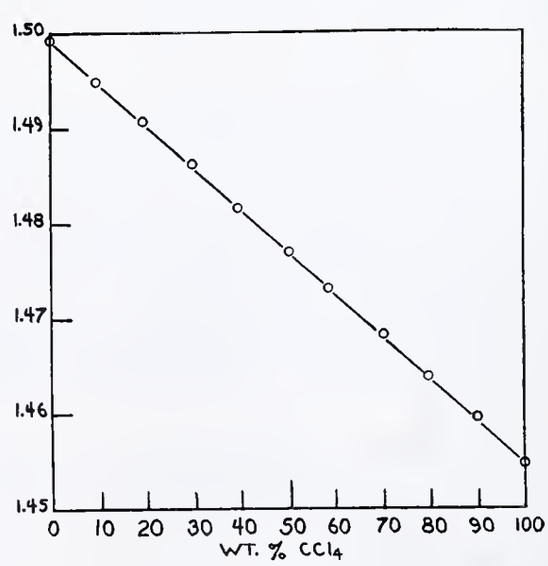

Figure 4. Refractive Index of System Carbon Tetrachloride–Tetrachloroethylene

Table V. Carbon Tetrachloride and Tetrachloroethylene

Carbon Tetrachloride, Weight %	Refractive Index at 25° C.
0	1.4993
9.85	1.4949
19.16	1.4907
29.65	1.4862
39.54	1.4818
49.75	1.4771
58.29	1.4733
69.94	1.4684
79.59	1.4640
89.94	1.4596
100.00	1.4549

the approximate 10 per cent divisions. When the results were plotted as refractive index against per cent by weight, it was found that a straight line fitted the data most accurately (Table V).

Summary

Two methods have been developed for the analysis of the ternary system, benzene–carbon tetrachloride–acetic acid, and one method for the binary system, carbon tetrachloride–tetrachloroethylene.

In the first method for the ternary system, the sample was weighed and the acid determined volumetrically with standardized sodium hydroxide solution, using thymol blue made up in a basic aqueous solution as the indicator. The phase containing benzene and carbon tetrachloride appears as the titration is carried out. The mixture was centrifuged and the composition of the benzene–carbon tetrachloride determined by refractive index methods.

The second method of analysis of the ternary system was based on measurements of density and refractive index. The density was determined by measuring the loss in weight of a drop of Pyrex brand glass when suspended in the liquids from a fine wire. Rigid temperature control over the mixtures was not necessary because change of density with change of composition was so great.

Refractive index measurements were used for the analysis of the binary system.

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Presented before the Division of Physical and Inorganic Chemistry at the 104th Meeting of the American Chemical Society, Buffalo, N. Y. Part of a thesis submitted by William R. McMillan in partial fulfillment of the requirements for the degree of master of science in the graduate school of the Illinois Institute of Technology.

Colorimetric Determination of Copper with 1,10-Phenanthroline

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TARTARINI (*5*) in a discussion of new color reactions involving cuprous salts, reported that 2,2′-bipyridyl and 1,10-phenanthroline form copper complexes which can be reduced with hydroxylamine to give highly colored cuprous compounds. The reaction is brought about by treating an ammoniacal solution of a cupric salt with the reagent and reducing. All cuprous salts containing ammonia or strong organic bases are either colorless or very pale. With a slight modification, the test with 1,10-phenanthroline can be used satisfactorily for the determination of copper.

Apparatus and Solutions

Transmittancy curves were determined with a General Electric recording spectrophotometer set for a spectral band width of 10 mμ.

A standard copper sulfate solution was prepared by dissolving copper wire in 1 to 1 nitric acid, fuming down with sulfuric acid, and diluting with redistilled water to a concentration of 0.05 mg. of copper per ml. Five milliliters of this solution diluted to 50 ml. give a copper concentration of 5 p. p. m.

A 0.1 per cent aqueous solution of 1,10-phenanthroline monohydrate was used as the color-forming reagent. The reducing agent was a 10 per cent solution of hydroxylamine hydrochloride. Both of these solutions are used in the 1,10-phenanthroline method for iron (*1*).

Commercial grades of the following solvents were used: acetone, ethanol, methanol, 2-propanol, dioxane, methyl Cellosolve, methyl Carbitol, ethyl Carbitol, chloroform, carbon tetrachloride, ethyl acetate, *n*-amyl alcohol, benzene, chlorobenzene, and ethyl ether.

pH adjustments were made with 6 *N* hydrochloric acid and 6 *N* ammonium hydroxide.

Standard solutions of the anions studied were prepared from the alkali metal salts. Nitrates, chlorides, and sulfates were used for the cations. Each solution contained 10 mg. of the ion in question per ml.

The Color Reaction

The order in which the reagents are added is of primary importance, the two following conditions being necessary: copper must be in the form of the ammonia complex and the reagent must be added before the hydroxylamine. Ammonia concentration is a critical factor but is easily controlled by neutralizing the solution to litmus and adding a measured quantity of 6 *N* ammonium hydroxide.

Formation of a complex cuprous salt such as $Cu(C_{12}H_8N_2)X$ is responsible for the orange color (*5*). The time required for precipitation to occur in aqueous solutions varies with the copper concentration. Precipitation is prevented entirely by the presence of certain alcohols or other solvents miscible with water. Other undesirable properties of aqueous solutions of the complex which can be avoided by addition of a solvent are instability of the color, nonconformity to Beer's law, and change of hue with copper concentration.

Forty per cent methyl Carbitol is most satisfactory of the solvents tried, although ethyl Carbitol can also be used. Fading occurs within 2 days with dioxane, methyl Cellosolve, or the alcohols. With methyl Carbitol, the slight change in 2 days is not measurable by visual means. Acetone reacts with the hydroxylamine.

Extraction of the color with any of the following solvents is not feasible: *n*-amyl alcohol, benzene, chlorobenzene, chloroform, carbon tetrachloride, ether, and ethyl acetate.

The 5-chloro, 5-bromo, 5-methyl, nitro, and 5-nitro-6-methyl derivatives of 1,10-phenanthroline have no advantage over the parent compound from the standpoint of sensitivity or stability of the colored complex. 2,2′-Bipyridyl is less sensitive and

2,2′,2″-terpyridyl does not react. In contrast, all of these compounds are about equally effective for determining ferrous iron (*1*, *4*).

Effect of Concentration of Reagents. The effects of concentrations of phenanthroline, ammonia, and hydroxylamine are not independent of one another. The effect of ammonia concentration, for example, is different for different concentrations of hydroxylamine. These variables can be easily controlled, however. For solutions with a final volume of 50 ml., 2 ml. of 6 *N* ammonium hydroxide, 10 ml. of 0.1 per cent phenanthroline solution, and 5 ml. of 10 per cent hydroxylamine hydrochloride are sufficient for copper concentrations up to 10 p. p. m. Concentration of methyl Carbitol is not critical. Twenty milliliters in 50 ml. of solution are recommended, but less may be used if precipitation of dissolved salts is likely to occur.

Buffering with ammonium acetate offers no advantage because the required amount of free ammonia must be present before reducing the copper, in which case proper adjustment of pH will have been made.

Effect of Copper Concentration. For a cell thickness of 1 cm., determinations of copper can be made conveniently in the range 0.1 to 10 p. p. m. as shown in Figure 1. All solutions contained 2 ml. of ammonium hydroxide, 8 ml. of reagent, 5 ml. of hydroxylamine hydrochloride solution, and 20 ml. of methyl Carbitol in 50 ml. The solution in the back cell was identical with the solution measured, except for copper, thereby canceling any adverse effects due to impurities in the reagents. Methyl Carbitol and several of the other solvents were found to contain considerable copper. Beer's law is valid at 435 mμ throughout the range of concentrations susceptible to measurement. The molecular extinction coefficient at 435 mμ is 7030. For visual work, standards such as the solutions represented in Figure 1 are reliable for at least 24 hours.

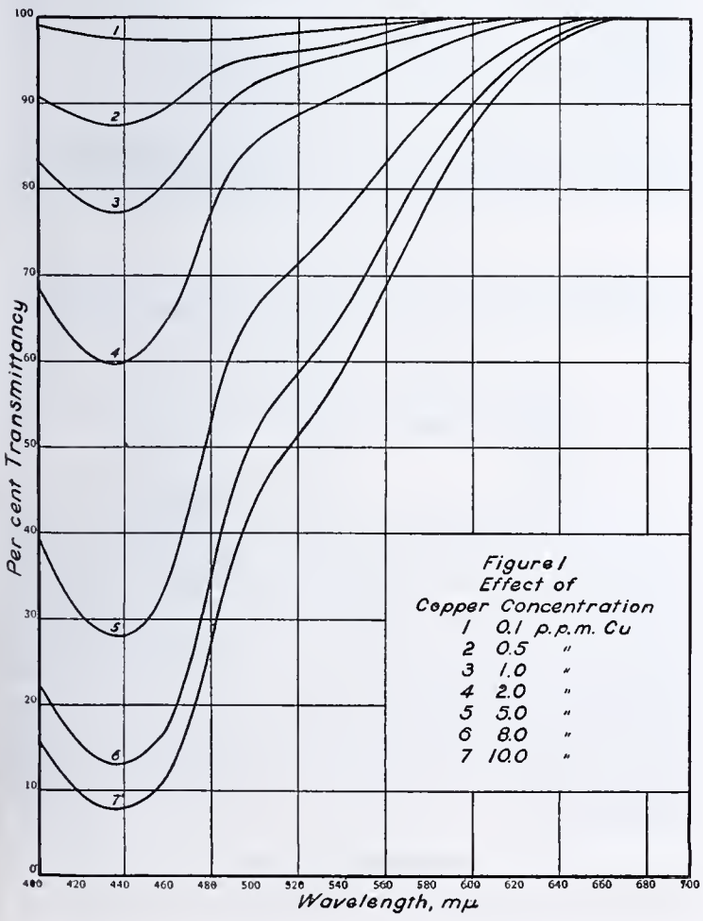


Table I. Effect of Diverse Ions

Ion	Added as	Present	Error	Amount Permissible
		P. p. m.	%	*P. p. m.*
Ba^{++}	$Ba(NO_3)_2$	500	3	300
Be^{++}	$Be(NO_3)_2$	100	0	100
Cd^{++}	$Cd(NO_3)_2$	10	2	10
Co^{++}	$Co(NO_3)_2$	10	6	5
Ni^{++}	$Ni(NO_3)_2$	5	2	5
Sr^{++}	$Sr(NO_3)_2$	500	10	100
Zn^{++}	$Zn(NO_3)_2$	10	2	10
CO_3^{--}	Na_2CO_3	500	3	300
CHO_2^-	HCO_2H	500	3	300
$C_2O_4^{--}$	$(NH_4)_2C_2O_4$	500	3	300
$C_3H_5O_3^-$	$CH_3CHOHCO_2H$	500	3	300
$C_4H_4O_6^{--}$	$(NH_4)_2C_4H_4O_6$	500	3	300
$C_6H_5O_7^{---}$	$(HO)C(CO_2H)(CH_2CO_2H)_2$	500	3	300
$C_7H_5O_2^-$	$C_6H_5CO_2Na$	500	3	300
$C_7H_5O_3^-$	$C_6H_4(OH)CO_2Na$	500	3	300
CN^-	KCN	2	14	0
$Cr_2O_7^{--}$ (Cr)	$K_2Cr_2O_7$	25	2	25
$P_2O_7^{--}$	$Na_4P_2O_7$	500	4	300
SCN^-	KSCN	500	6	250
$S_2O_3^{--}$	$Na_2S_2O_3$	30	18	5
VO_3^-	KVO_3	500	14	100

Effect of Diverse Ions. The phenanthroline method for copper is comparatively free from interference by the anions. Few cations interfere aside from those which precipitate in the presence of ammonia.

In determining the extent of interference for the various ions, the following procedure was adopted: A measured volume of solution containing the ion in question was added to 5 ml. of the copper sulfate solution containing 0.05 mg. of copper per ml. After neutralizing to litmus with 6 *N* ammonium hydroxide or hydrochloric acid, the following were added in order: 2 ml. of 6 *N* ammonium hydroxide, 10 ml. of reagent solution, 1 ml. of hydroxylamine solution, and 20 ml. of methyl Carbitol. The solution was then diluted to 50 ml. with redistilled water and the transmission curve run. Ammonium acetate was added in a number of cases without any apparent advantage. If interference was pronounced, smaller quantities of the ion were used successively until an estimate of the limiting permissible concentration could be made, 2 per cent error being set arbitrarily as a reasonable tolerance. These values are summarized in Table I.

For solutions containing 5 p. p. m. of copper, the following ions may be present in concentrations of at least 500 p. p. m. without causing an error greater than 2 per cent: ammonium, calcium, lithium, magnesium, potassium, sodium, acetate, arsenate, arsenite, bromide, chlorate, chloride, fluoride, iodate, iodide, molybdate, nitrate, nitrite, orthophosphate, perchlorate, periodate, silicate, sulfate, sulfite, tetraborate, and tungstate.

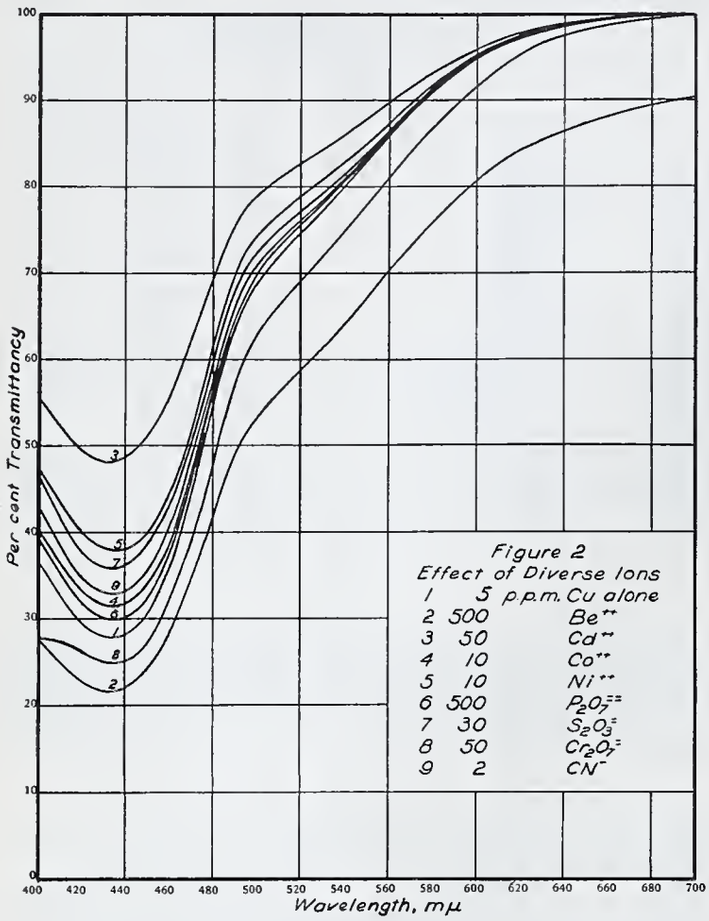
The following cations precipitate: aluminum, antimony, bismuth, cerium, chromium, cobalt, iron, lead, manganese, mercury, silver, titanium, thorium, uranium, zirconium, and beryllium (in concentrations exceeding 100 p. p. m.).

Cadmium, cobalt, nickel, and zinc interfere presumably by complex formation with the reagent. These metals also interfere in the determination of iron with 1,10-phenanthroline (*1*).

Among the anions, serious interference was encountered only with cyanide and thiosulfate. These should be absent. Large amounts of dichromate and metavanadate also interfere. Transmission curves for several solutions containing interfering ions are shown in Figure 2.

Discussion

Since the color reaction must be carried out in ammoniacal solution, iron must be removed irrespective of the fact that it, also, gives an intense color with 1,10-phenanthroline. This separation can be made by careful precipitation with ammonia as directed by Mehlig (*3*). When the filtration has



been completed, copper is present as the ammonia complex and, if the concentration is high enough to give a measurable color, no advantage over the ammonia method (*3*) can be gained by using 1,10-phenanthroline. If the concentration is too low, however, the proposed method may be used with good results for concentrations greater than 0.1 p. p. m.

In using the method for copper ores, results were calculated by the following procedure.

Instead of comparing the transmittancy of the unknown solution with a standard, the change in transmittancy produced by adding a known amount of copper to the unknown was determined. The unknown concentration, x, was then calculated according to Beer's law expressed as follows:

$$T_x = T_{a+x}^{\frac{x}{a+x}}$$

$$x = \frac{a \log T_x}{\log \frac{T_{a+x}}{T_x}}$$

in which T_x is the transmittancy of an aliquot of the sample and T_{a+x} the transmittancy of a similar aliquot containing a small additional quantity of copper, a. Uncertainties arising from the presence of other constituents, turbidity, extraneous color, or other sources, except presence of copper in the reagents, are thus compensated. Foster, Langstroth, and McRae have applied this principle successfully to the spectrographic determination of lead (*2*).

1,10-Phenanthroline is somewhat less sensitive as a copper reagent than is sodium diethyldithiocarbamate. The latter reagent forms an insoluble copper salt requiring use of gum arabic or extraction with a suitable solvent. Methyl Carbitol is not satisfactory for preventing precipitation with the diethyldithiocarbamate method nor can gum arabic be used with the phenanthroline method.

Recommended Procedure

Treatment of Sample. Prepare a solution of the sample by appropriate means to contain 0.01 to 0.5 mg. of copper (see *3* for copper ores and mattes).

If necessary to remove any metals precipitated by ammonia, make the solution just basic to litmus by adding 15 *N* ammonium hydroxide, filter into a conical flask, and wash the precipitate with 3 *N* ammonium hydroxide until the washings emerge colorless. Remove the flask, wash the precipitate into the original container, and dissolve the material with a minimum amount of concentrated sulfuric acid. Then repeat the precipitation and washing with ammonium hydroxide as before, and catch the filtrate in the same conical flask. Evaporate the solution to 15 ml.

Measurement of Desired Constituent. Neutralize the solution to litmus with 6 *N* hydrochloric acid or ammonium hydroxide and add the following in order: 2 ml. of 6 *N* ammonium hydroxide, 10 ml. of 0.1 per cent 1,10-phenanthroline solution, 1 ml. of 10 per cent hydroxylamine hydrochloride solution, and 20 ml. of methyl Carbitol.

Dilute to 50 ml. with water, mix well, and measure or compare the color by any of the usual means. A blue filter such as Corning No. 556 is recommended for filter photometers.

Summary

The intense, brown color of cuprous-phenanthroline complex may be used as the basis of a colorimetric method for the determination of copper. Beer's law is valid for concentrations from 0.5 to 10 p. p. m. of copper, at least, this being the range most suitable for measurements with a 1-cm. transmission cell. The colored system is stable for at least 24 hours.

Of the metals whose compounds are soluble under the conditions used, only cadmium, cobalt, nickel, and zinc interfere seriously. Metals which precipitate with ammonia are removed during the course of the procedure. Among the anions studied, only cyanide, dichromate, and thiosulfate interfere appreciably.

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Modification of Calibration Apparatus

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SEVERAL minor improvements in precision calibration apparatus (*1*) have been introduced, and the stopcock-control chamber has been redesigned. Stopcock *E* is eliminated, as is clamp *H*, and instead stopcocks are added just above *A* and *B*. These and *D* are used to control flow rates, and the inner set, *A*, *B*, *C*, and *F*, are used as open or shut gates, as in previous procedure. Control is made easier and elimination of entrapped air after greasing a stopcock may now be accomplished without tilting the apparatus. Furthermore, a vessel of smaller capacity but otherwise the same pattern as vessel *W* of Figure 3 is preferable for calibration of microburets.

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Spectrographic Detection and Determination of the Halogens

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THE spectrochemical analysis of the halogens has been confined, with one exception, to the detection and determination of fluorine as calcium fluoride (*1, 5, 7*) or silicon fluoride (*6*). Pfeilsticker (*8*) has analyzed salts for bromine, chlorine, and iodine at reduced pressures by the use of a special, very sparklike spark (2000-microfarad condenser, 0.05–0.1 millihenry inductance, at 220 volts). Nevertheless, the intensity of the spark lines of the halogens tabulated by Exner and Haschek (*2*) suggested strongly that it would be possible to detect all the halogens spectrographically in the usual spark source, if it were not for the presence of air lines, principally of nitrogen. The air lines could not be quenched by the usual expedient of introducing self-inductance into the spark circuit because the sparklike qualities of the source would thereby be degraded toward the arc, and the spark lines of the halogens would become exceedingly faint. Despite this difficulty, it seemed feasible to determine the halogens spectrographically without the use of special auxiliary equipment and this possibility was investigated.

All spectrograms were taken in the first order of a 3-meter grating spectrograph, dispersion 5.6 Å. per mm. Eastman 33 plates were used for determining chlorine and bromine; Eastman spectroscopic I-F plates for fluorine and iodine. Overlapping higher order spectra were eliminated by suitable Corning glass filters placed before the spectrograph slit. The finely divided samples were introduced into the cored crater of 0.6-cm. (0.25-inch) graphite electrodes (National Carbon Co., Acheson grade) and flattened off level with the rim of the crater. The loaded electrode was placed in the lower clamp of a spark stand. A sharply pointed electrode was placed in the upper clamp of the spark stand and so adjusted that its point was exactly 2 mm. above the surface of the sample. The electrodes were connected to the terminals of a conventional spectrographic, alternating current, condensed-spark circuit with the following constants: input, 130 volts; output, 25,000 volts, 17 to 21 milliamperes; capacity 0.004 microfarad. The large inductance customarily used in such circuits to eliminate the air lines was shorted out, so that the only inductance was the natural self-inductance of the circuit. All leads were made as short as practicable. The spark was focused on the slit of the spectrograph by a simple quartz lens.

The following spectral lines have proved to be the most satisfactory for the detection and determination of the halogens:

Element	Wave Length Å.	Intensity	Element	Wave Length Å.	Intensity
F	6856.02	60	Cl	4794.54	20
	6902.46	40		4810.06	10
				4819.46	8
Br	4678.69	10			
	4704.86	30	I	5161.19	50
	4785.50	15		5464.61	30
	4816.71	10			

The wave lengths are taken from the M. I. T. Wavelength Tables (*4*). The intensity estimates are the authors' and are based on the intensity scale of 1 to 1000 used by Exner and Haschek in their measures of the air lines (*2*).

Air lines did not interfere with any of the strongest lines of fluorine and iodine, but all the chlorine lines except 4794.54 Å. were masked by nitrogen lines, as was the strongest line of bromine. The masking of these sensitive lines reduced the sensitivity of the spectrographic test for chlorine and bromine very considerably.

To overcome the interference of the air spectrum, the electrodes were introduced inside a 5-cm. diameter Pyrex tube through the center of two rubber stoppers (the interelectrode gap was still maintained at 2 mm.). Off-center holes in each stopper held 3-mm. diameter glass tubes. Carbon dioxide was introduced through the lower tube and escaped through the upper tube. A few ounces of carbon dioxide pressure sufficed to sweep the tube clear of nitrogen in less than a minute. It was, of course, necessary to use pure carbon dioxide. Carbon dioxide generated from dry ice was found to be contaminated with chlorine, and ordinary commercial carbon dioxide contained traces of nitrogen and chlorine. Through the courtesy of E. N. Ellis of the S. S. White Dental Mfg. Co. of Philadelphia, the authors obtained U. S. P. carbon dioxide customarily used in anesthesia. No nitrogen or chlorine could be detected in this gas by spectrographic methods.

The spectrographic sensitivity of the halogens varied with the matrix in which they were found and the cations to which they were attached. In general, amounts of the following orders of magnitude could be detected with certainty:

Element	Limit: Identification *Micrograms*	Limit: Sensitivity %
Br in CO_2	500	0.3
Cl in CO_2	900	0.5
F in air	200	0.1
I in air	100	0.07

where the limit of identification refers to the quantity sensitivity, and the limit of sensitivity refers to the concentration sensitivity, in accordance with the definitions given by Feigl (*3*). One half these limiting amounts could be detected in many materials, one fourth these amounts in some materials.

Satisfactory quantitative determinations of chlorine were carried out in the carbon dioxide atmosphere in the range from 1 to 15 per cent of chlorine when 150 mg. of sample, introduced in five separate loads, were sparked. The accuracy of the determinations varied from ±10 per cent of the amount of chlorine found when the chlorine content was less than 5 per cent to ±5 per cent when the chlorine content was 15 per cent. The precision of the determinations was uniformly of the order of ±5 per cent.

Summary

Materials containing the halogens were sparked without prior chemical treatment in the usual high-voltage spectrographic spark. By this method the halogens could be determined with an accuracy comparable to that obtained in the spectrochemical analysis of metals. In the case of chlorine and bromine the most sensitive lines were masked by air lines and observations were carried out in a carbon dioxide atmosphere in a simple cylindrical tube. The limits of detection were 0.5 per cent for chlorine and 0.3 per cent for bromine, in a carbon dioxide atmosphere; 0.1 per cent for fluorine and 0.07 per cent for iodine, in air.

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Cerate and Periodate Oxidimetry

Perchlorato-Cerate and Periodate Ions as Oxidants in the Determination of Organic Compounds

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THE most satisfactory general procedures for the quantitative determination of organic compounds are those of Stamm (*5*) and Malaprade (*2*). The Stamm procedure involves oxidation by the permanganate ion in alkaline solution. The practical difficulties in its application make its use unusually troublesome. The Malaprade procedure, in which periodic acid is used as the oxidant, is not so generally applicable but is much to be preferred over the Stamm procedure.

The perchlorato-cerate ion, $Ce(ClO_4)_6^{--}$, in perchloric acid solution, has been employed by Smith and Duke (*4*) in the determination of glycerol, and the same type of procedure for the determination of an extensive series of organic compounds is described in the present work. The applications described are limited to oxidations in acid solution, and, in general, to aliphatic organic compounds. The results are stoichiometric rather than empirical, the reactions are rapidly complete at ordinary temperatures, and oxidation-reduction indicators are applicable throughout.

In this procedure, as well as in the Stamm and Malaprade procedures, the determination is not specific or selective in its action without supporting analyses of the reaction products obtained with variations of reaction conditions.

A bibliography of previous work on the general subject of the quantitative oxidation of organic compounds has been given by one of the present authors (*3*).

Oxidation of Organic Compounds Using Periodic Acid

A typical oxidation of an organic compound using periodic acid may be illustrated by citing the determination of glycerol as described by Fleury and Fatome (*1*). The reactions are as follows:

$$CH_2OH.CHOH.CH_2OH + 2Na_3H_2IO_6 + 2H_2SO_4 \rightarrow 2HCHO + HCOOH + 2NaIO_3 + 2Na_2SO_4 + 5H_2O$$

$$Na_3H_2IO_6 + Na_3AsO_3 + 2NaHCO_3 \rightarrow Na_3AsO_4 + NaIO_3 + 2Na_2CO_3 + 2H_2O$$

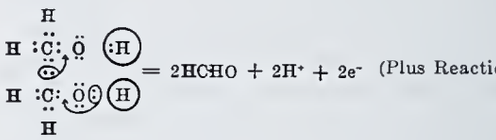
The oxidation of glycerol in dilute sulfuric acid solution is complete in 5 minutes at ordinary temperatures with an excess of periodate. An excess of sodium bicarbonate is added and the excess of periodate reduced, using excess of standard arsenite solution. The excess arsenite is then determined using standard iodine solution with starch as indicator. The presence of iodate from the reduction of periodate does not interfere.

The principles involved in the oxidation of various types of organic compounds which may be determined by a series of reactions similar to those of the determination of glycerol using periodate, will be apparent from the following discussion of the Malaprade procedure.

Two electrons and two protons are removed from organic compounds containing hydroxyl groups in the alpha position to each other (the ethylene glycol type of structure). The electronic structure of the remainder of the molecule is unstable and must be capable of rearrangement to a stable configuration.

In the case of organic compounds having two aldehyde groups, an aldehyde and alcoholic group, or an aldehyde and keto group in the alpha position to each other, each pair of groups hydrates and is thus converted into the ethylene glycol type of configuration and oxidized in a similar manner.

Formaldehyde, acetaldehyde, propionaldehyde, etc., are oxidized by periodic acid only after an extended period of time. Illustrative reactions are those of the oxidation of ethylene glycol, glyoxal, glycollic aldehyde, and pyruvic aldehyde.



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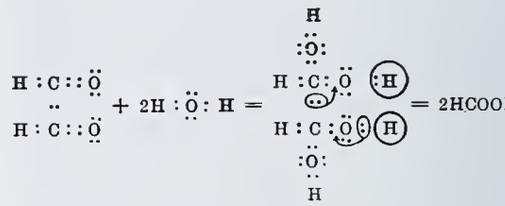

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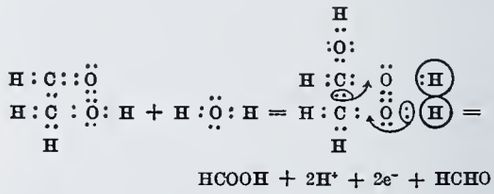

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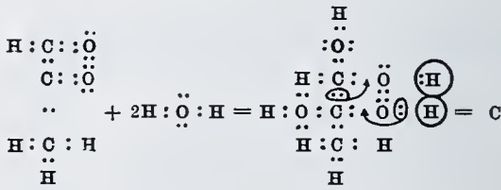

IV

Polyhydroxy compounds in general are oxidized using excess of periodate—for example, ethylene glycol, erythritol, arabitol, and mannitol. These reactions are summarized in the following general formulation:

$$CH_2OH.(CHOH)_n.CH_2OH + (n + 1)HIO_4 = (n + 1)\ HIO_3 + n(HCOOH) + 2HCHO + H_2O$$

In the case of polyhydric dicarboxylic acids in which n is greater than 1, such as tartaric acid, trihydroxyglutaric acid, and saccharic acid, the general formulation is

$$COOH.(CHOH)_n.COOH + (n - 1)HIO_4 = (n - 2)HCOOH + 2CHO.COOH + (n - 1)HIO_3$$

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Table I. Composite Results in Determination of Organic Compounds by the Perchlorato-Cerate–Oxalate Procedure

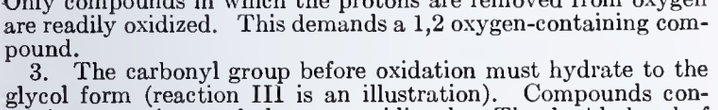
Sample	Sample Weight	$Ce(ClO_4)_6^{--}$		$C_2O_4^{--}$		No. of Determinations	Temp.	Time	Results Found	Accuracy	Equivalents Required
	Mg.	*Ml.*	*N*	*Ml.*	*N*		*° C.*	*Min.*	*Mg.*	*Av. %*	
Glycerol	23.90	25.00	0.1036	4.952	0.1032	4	45	15	23.925	+0.10	8
Glucose	27.54	25.00	0.1040	6.940	0.1036	5	26	45	27.580	+0.15	12
Sucrose	59.88	50.00	0.1040	6.302	0.1038	5	24	45	59.920	+0.07	26
Cellulose	29.72	50.00	0.1035	32.272	0.1035	5	27	120	29.774	+0.18	12
Biacetyl	171.4	75.00	0.0927	28.00	0.1050	1	24	5	171.8	+0.4	2
	83.6	25.00	0.0927	3.75	0.1050	1	24	5	82.7	−0.6	2
	49.1	25.00	0.0927	11.12	0.1050	1	24	5	49.1	0.0	2
	504.9	150.00	0.0927	20.54	0.1050	1	24	5	504.9	0.0	2
Acetyl acetone	100.83	100.00	0.1043	41.578	0.1056	4	25	10	100.92	+0.09	6
Tartaric acid	88.15	50.00	0.1032	22.775	0.1036	4	26	10	88.225	+0.08	6
Malonic acid	104.32	100.00	0.1032	41.605	0.1036	4	26	10	104.375	+0.05	6
Citric acid	44.925	50.00	0.1032	18.173	0.1036	4	10	30	44.825	0.00	14
Malic acid	107.0	100.00	0.1032	38.205	0.1036	4	25	15	107.075	+0.02	8

These two reactions indicate that tartaric acid requires 6 equivalents for oxidation and mannitol 10 equivalents. All the periodic acid oxidations require an even number of equivalents and are thus not empirical in any instance.

Oxidation of Organic Compounds by Perchlorato-Ceric Acid in the Presence of Perchloric Acid

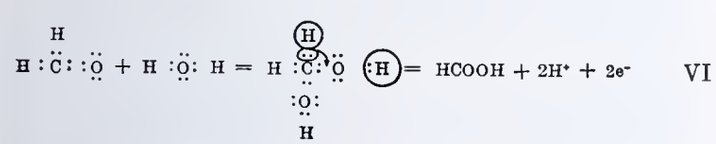
The principles governing the oxidation of aliphatic organic compounds by use of perchlorato-ceric acid in the presence of 4 molar perchloric acid are as follows:

1. In each case an even number of equivalents of oxidant is required. End products are fatty acids, ketones, aldehydes (other than formaldehyde), and carbon dioxide. In all cases the oxygen demand is stoichiometric rather than empirical. All reactions are carried out at room temperature or at slightly elevated temperatures only.
2. The simplest type of oxidation follows the direct liberation of two protons and two electrons (reaction I + II is an illustration). Only those compounds, the electronic configuration of which is capable of rearrangement to a stable form after the removal of two protons and two electrons, are oxidizable. Only compounds in which the protons are removed from oxygen are readily oxidized. This demands a 1,2 oxygen-containing compound.
3. The carbonyl group before oxidation must hydrate to the glycol form (reaction III is an illustration). Compounds containing an active methylene are oxidizable. The double bond of the enol, formed by such active methylene group, is saturated by hydroxyl groups resulting from reaction V.



V

4. Formaldehyde is hydrated and oxidized with the consumption of two oxygen equivalents in accordance with reaction VI.

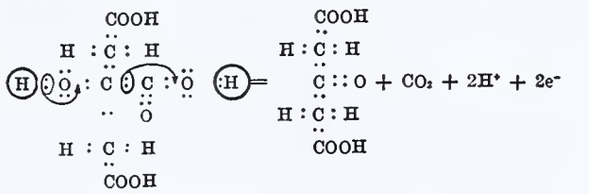


VI

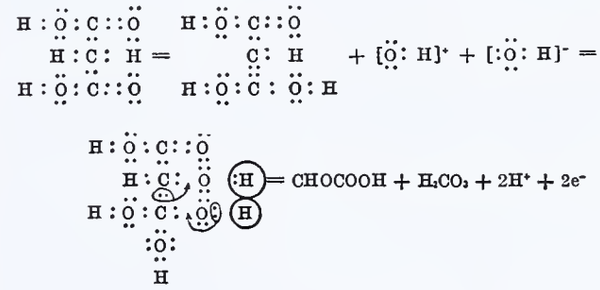
In this case one proton is detached from direct union to carbon, a specific property of cerate oxidimetry as distinct from periodate oxidimetry. The reaction is rapid. In case aldehydes other than formaldehyde are involved or the ketone group is present unsubstituted by oxygen in the alpha position, either no reaction results under the condition of the experiment or empirical results are obtained.

5. Oxalic acid is instantly oxidized at ordinary temperatures. This reaction is used in the determination of excess perchlorato-cerate ion. Nitro-ferroin is used as internal indicator for this reaction. The standardization of the perchlorato-cerate solution in this case should be carried out by titration with standard oxalate rather than by the reverse procedure.

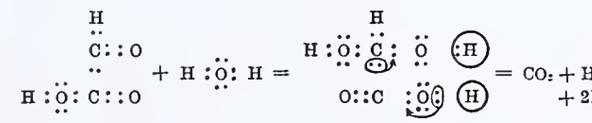
The above rules are all illustrated in the oxidation of citric acid developed in reactions VII, VIII, IX, and X.



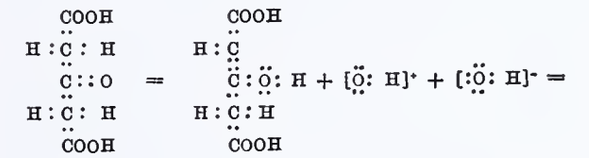
VII



VIII



IX



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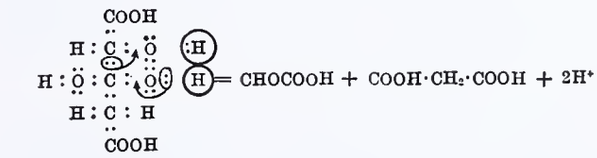

The citric acid is first oxidized to acetone dicarboxylic acid (reaction VII) with the consumption of 2 equivalents. The enolized form of the acetone dicarboxylic acid then has the double bond broken with the consumption of two more equivalents, as illustrated by reactions V and VIII, forming 1,3-dicarboxy-2,2,3-trihydroxypropane, which is oxidized by 2 more equivalents to form one mole of malonic acid and one mole of glyoxylic acid. The glyoxylic acid is then hydrated and oxidized to formic acid with the consumption of 2 more equivalents, according to reaction IX. Lastly, the mole of malonic acid enolizes and the double bond is broken by the reaction of the positive and negative hydroxyl, accounting for the use of 2 additional equivalents of oxidant and forming another molecule of glyoxylic acid (reaction X). Lastly, two more equivalents of oxygen are required to oxidize this glyoxylic acid in accordance with reaction IX. The

total number of oxygen equivalents demanded is 14. The total reaction is shown as follows:

$$C_6H_8O_7 + 14Ce(ClO_4)_6^{--} + 5H_2O = 4CO_2 + 2HCOOH + 84ClO_4^- + 14H^+ + 14Ce^{+++}$$

The formulas for the group oxidation of various type reactions are given in the following summary:

XI. Polyhydric Alcohols

$$CH_2OH.(CHOH)_n.CH_2OH - (2n + 6)e^- + (n + 2)H_2O = (n + 2)HCOOH + (2n + 6)H^+$$

XII. Polyhydric Alcohol Monocarboxylic Acids

$$CH_2OH.(CHOH)_n.COOH - (2n + 4)e^- + (n + 1)H_2O = (n + 1)HCOOH + (2n + 4)H^+ + CO_2$$

XIII. Polyhydric Dicarboxylic Acids

$$COOH.(CHOH)_n.COOH - (2n + 4)e^- + nH_2O = nHCOOH + 2CO_2 + (2n + 4)H^+$$

XIV. Aldoses

$$CH_2OH.(CHOH)_n.CHO - (2n + 4)e^- + (n + 2)H_2O = (n + 2)HCOOH + (2n + 4)H^+$$

XV. Ketoses

$$CH_2OH.(CHOH)_n.CO.CH_2OH - (2n + 8)e^- + (n + 3)H_2O = (n + 2)HCOOH + (2n + 8)H^+ + CO_2$$

Conditions Governing Experimental Reaction Procedures

For the quantitative and stoichiometric oxidation of organic compounds several requirements must be fulfilled. Each organic molecule must react to give definite end products and all side reactions must be excluded. The end products must not be further oxidized under the defined experimental conditions. A time limit for reaction has been established of 120 minutes or less. Any reaction for which an odd number or fractional number of equivalents are employed is known to be empirical and unsatisfactory.

Solutions of perchlorato-ceric acid in 4 *N* perchloric acid may be heated at 60° C. for 60 minutes without appreciable loss in titer due to the oxidation of water (reaction V). Formic acid is not appreciably oxidized by the same cerate solution, at the same temperature, in the same period of time. Amounts of acetic acid experimentally encountered may be present without appreciable error during periods of 60 minutes at 50° C. With acetaldehyde and acetone present as products of the reaction of oxidation, more than 5 minutes at 10° C. introduces appreciable error. These values were established by a procedure duplicating that described by the present authors (*4*) for the case of the determination of glycerol.

Testing the general procedure applicable in specific instances consisted in the addition of known amounts of the various organic compounds to 4 *N* perchloric acid solutions containing about a twofold excess of perchlorato-cerate ion. The reaction was allowed to develop at a definite temperature for specified periods of time and at the end the reacting solution was diluted by the use of an equal volume of water. A drop of 0.025 *M* nitro-ferroin indicator was added and the excess perchlorato-cerate ion was titrated, using standard sodium oxalate solution, until the first drop of excess produced a faint pink solution. The reduction of excess oxidant was carried out at ordinary temperatures.

The end products were either qualitatively detected or predicted by analogy, and the experimental conditions were limited to those permissible in the presence of the known end products. If the oxygen equivalent calculated did not agree with that experimentally found, side reactions were indicated demanding altered conditions or elimination of the procedure from practical application.

The results of the analyses of a series of pure organic compounds are given in Table I.

Summary

The mechanism of quantitative oxidation of organic compounds using periodic acid as oxidant has been described. The same mechanism has been applied to the oxidation of organic compounds using the perchlorato-cerate ion, $Ce(ClO_4)_6^{--}$, in the presence of 4 *M* perchloric acid. The latter procedure has been studied for a considerable number of individual cases and the type reactions involved make possible its application to a more extensive series of compounds.

The use of the perchlorato-cerate ion and periodate ion in these procedures follows the same general scheme. The organic compound is oxidized by excess of oxidant in acid solution for a specified time at a given temperature, after which the excess oxidant is evaluated using a standard reducing agent. The use of periodic acid is more complicated than that of the perchlorato-cerate ion because the excess periodate ion must be determined in the presence of the iodate ion, necessitating the use of an added standard solution. The perchlorato-cerate ion is more extensive in its applicability, the number of oxidation equivalents required is larger, and the speed of the reactions in general greater than in the case of the periodate ion.

All reactions by both procedures are stoichiometric rather than empirical, and an exceptional degree of accuracy is obtainable in all cases. The methods apply to aliphatic compounds and are not specific in their action but require special treatment to isolate the substance to be determined if interfering substances are present.

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Thimble Supports for Faster Soxhlet Extraction

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THE insertion of a piece of glass rod at an angle into the extractor (midpiece) of a Soxhlet apparatus to raise the thimble off the bottom, thus securing better drainage and a more rapid rate of extraction, has recently been recommended (*1*). The author has occasionally used this and similar methods in the past, particularly where it was necessary to use a thimble shorter than the height of the siphon, so that undissolved matter might have been displaced from the thimble mechanically if the liquid had been allowed to rise above the top of the thimble.

However, an oblique glass rod was not always satisfactory as a thimble support. When inserting or removing the thimble, there was sometimes a tendency for the rod to shift into such a position that its lower end obstructed the outlet of the siphon, thus impeding or preventing proper extraction.

If this trouble is encountered, a ground-glass stopper with a flat or mushroom-type top may be inverted in the bottom of the extractor to support the thimble without obstructing the siphon. To avoid breakage, it should be carefully inserted or removed with crucible tongs or forceps. By somewhat diminishing the volume of liquid which can collect at each filling, the glass stopper promotes more frequent siphoning and faster extraction. A weighing bottle, or large homeopathic vial, of appropriate height and diameter, inverted in the extractor, also serves as an effective thimble support in some cases.

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Thiocyanogen Absorption of Linseed Oils

Thiocyanogen Absorption of Linoleic and Linolenic Acids and Composition of Linseed Oils

EDGAR PAGE PAINTER AND L. L. NESBITT, North Dakota Agricultural College, Fargo, N. D.

IN CONNECTION with studies of fat metabolism in flaxseed and of the fat acid distribution responsible for the wide variations in the degree of unsaturation of linseed oils, it became necessary to determine the composition of a large number of oils. Before undertaking this work it seemed desirable to apply to linseed oil some of the refinements of technique recently shown to be necessary for the analysis of mixtures of oleic, linoleic, and linolenic acids.

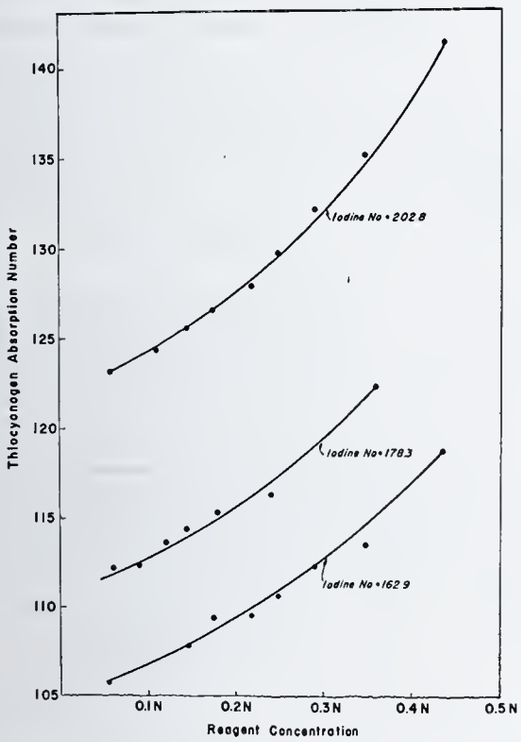

FIGURE 1. EFFECT OF REAGENT CONCENTRATION ON THIOCYANOGEN ABSORPTION OF LINSEED OIL

Approximately same amount of reagent used for all points of each curve.

Except for the composition of three linseed oils reported by Hilditch and Murti (*7*) and of seven by Rose and Jamieson (*25*), nearly all analyses of linseed oils have been based on calculations using Kaufmann's theoretical constants (*11, 12*) for the thiocyanogen absorption numbers of linoleic and linolenic acids. Kimura (*16*) in 1929 presented evidence which placed Kaufmann's theoretical constants in doubt. Nevertheless, they were widely used until recently when several laboratories investigated the validity of Kaufmann's values. As a result of their work (*7, 9, 10, 19, 22, 23, 26*) the thiocyanogen values have been modified, so that in using the method originally proposed by Kaufmann one can now obtain results which are much more accurate than previously possible.

Thiocyanogen absorption numbers of oleic, linoleic, and linolenic acids and esters determined by several groups of investigators have been summarized by Riemenschneider, Swift, and Sando (*22*). Matthews, Brode, and Brown (*19*) have since reported values for their carefully purified specimens of these acids. The results obtained by the several groups are in fair agreement, but it is clear that the absorption of thiocyanogen depends upon the conditions under which the determination is made. The concentration of reagent (*9, 10, 22*), the excess of reagent over that added to the ethylenic bonds (*9, 10, 19, 22, 23*), the length of time permitted for addition of the reagent (*9, 10, 19, 22*), the temperature of the reaction (*19, 22*), and the solvent (*19*) are factors which alter the thiocyanogen number of linoleic and linolenic acids. Oleic acid, on the other hand, gives nearly theoretical thiocyanogen absorption numbers by most procedures.

In order to obtain accurate results it is imperative that thiocyanogen should add to the double bonds of the fat acids of the oil in the same ratio that it adds to the fat acids when the empirical constants are determined. It therefore seemed necessary to determine those factors which must be controlled to obtain precise thiocyanogen absorption constants of linseed oil, and the empirical constants for the thiocyanogen absorption of linoleic and linolenic acids by following the procedure finally adopted for linseed oil.

Thiocyanogen Absorption of Linseed Oil

To obtain the data shown in Figure 1, equal volumes of the most concentrated solution used with each oil were diluted with acetic acid reagent to the weaker concentrations. It can be seen that the concentration of the thiocyanogen reagent must be held within narrow limits if reproducible results are to be obtained. The thiocyanogen number is approximately 3 points higher with the 0.2 *N* solution recommended by the American Oil Chemists' Society (*1*) than with the 0.1 *N* solution recommended by the A. O. A. C. (*2*). The higher iodine number oil showed a greater increase in the thiocyanogen number with increase in reagent concentration than the lower iodine number oil. This is readily explained by the larger amount of linolenic acid in the former (Table VI).

The rate of addition of thiocyanogen to linseed oil (Figure 2) follows closely the rate predicted from studies of oleic (*23*), linoleic (*17, 23*), and linolenic (*9, 19*) acids. Thiocyanogen continues to add beyond 24 hours, but the rate of addition is slow enough so that this appears to be a suitable reaction time. These results differ markedly from those of Gay (*5*), who found that the thiocyanogen number of linseed oil increased rapidly up to about 48 hours, and from those of Kaufmann

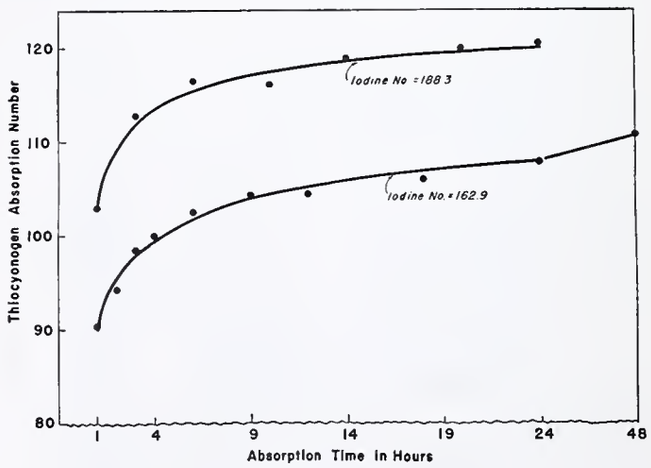

FIGURE 2. RATE OF ADDITION OF THIOCYANOGEN TO LINSEED OILS

Table I. Effect of Excess of Thiocyanogen Reagent

Oil 11		Oil 18		Oil 19		Oil 21		Oil 22	
Thiocyanogen No.	Excess thiocyanogen %	Thiocyanogen No.	Excess thiocyanogen %	Thiocyanogen No.	Excess thiocyanogen %	Thiocyanogen No.	Excess thiocyanogen %	Thiocyanogen No.	Excess thiocyanogen %
106.6	51	125.1	55	114.5	63	117.2	53	112.1	129
106.0	63	125.4	95	115.5	96	119.0	123	113.5	204
106.7	107	127.2	172	114.6	115	120.9	192	114.7	209
107.4	130	126.4	183	115.6	204	120.9	201	114.2	377
107.0	214	126.6	293	114.7	306	121.7	259	...	...
107.8	234	...	...	...	...	...	...	...	...
108.4	271	...	...	...	...	...	...	...	...
107.8	292	...	...	...	...	...	...	...	...

Iodine No.: oil 11, 162.9; oil 18, 202.8; oil 19, 178.2; oil 21, 188.3; oil 22, 176.6.

and Keller (*13*) and Griffiths and Hilditch (*6*), who found no increase after 18 hours.

In Table I, thiocyanogen numbers of several linseed oils obtained by varying the excess of thiocyanogen reagent are shown. The original concentration of reagent was approximately the same in each case. The excess reagent is expressed as $\frac{\text{amount unused}}{\text{amount added to double bonds}} \times 100$. These data indicate that the thiocyanogen numbers increase slightly with a greater excess of reagent, but the increment of increase is small. Over much of the range studied the differences are about the same as expected from the precision of the thiocyanogen determination (see Table II). We can expect somewhat better precision for the results in Table I because, with the exception of a few repeat determinations, the thiocyanogen values for each oil were determined with the same solution.

The extent of thiocyanogen addition to double bonds should be determined by the concentration of thiocyanogen and not by total unused reagent. In actual practice sufficient time is not allowed for the reaction to reach equilibrium, and the initial rate of addition may also be modified. Nevertheless, the authors believe the "effect of excess reagent" is primarily due to the concentration at the time the thiocyanogen not added to the double bonds is titrated. Relatively large differences in the per cent of excess reagent cause relatively small changes in thiocyanogen concentration. With a solution of original 0.2 N concentration and percentages of excess of 50, 100, 200, 300, and 400, the corresponding thiocyanogen concentrations are 0.067 N, 0.10 N, 0.133 N, 0.15 N, and 0.16 N. By increasing the excess reagent above 200 per cent the increased absorption would probably be small, if not negligible.

Nearly all of the authors' determinations on linseed oil which have been used to calculate composition have had 180 to 230 per cent excess reagent. The average has been 206 per cent. Within this range and with this great an excess, discrepancies due to the quantity of reagent have been largely eliminated.

One of the objections often raised to the use of thiocyanogen numbers for the calculation of the composition of fat acids is the difficulty in obtaining reproducible results. Soon after Kaufmann originally proposed the thiocyanometric method some workers concluded it was not dependable. After the several variables now known to change the thiocyanogen number were rigidly controlled, the discrepancies largely disappeared in most laboratories. The authors have determined the thiocyanogen number of a reference oil sample with each new 0.19 N thiocyanogen solution prepared (Table II). The maximum variations are larger than desired. These values, however, show better agreement than many results on purified samples of fat acids and esters where several determinations on the same samples are reported. The data in Table II include the thiocyanogen numbers (average of duplicate determinations) of each solution and not a single determination on the reference samples was excluded. In practice the precision of the method is better than the data in Table II indicate. When the iodine number of linseed oil is plotted against the thiocyanogen number, most of the points fall close to a straight line. Whenever a determination gives a point several units from this line, it is repeated. Almost without exception the repeat value falls very close to the line. Recently the authors have routinely determined the thiocyanogen number of each oil sample with two or more solutions. Numerous repeats have been necessary and occasionally one of duplicate determinations is out of line. Nevertheless, they believe very few of their values deviate more than 1 point from the "true" thiocyanogen number.

Thiocyanogen Absorption of Linoleic Acid, Methyl Linoleate, Linolenic Acid, and Methyl Linolenate

The authors have prepared several samples of linoleic acid, methyl linoleate, linolenic acid, and methyl linolenate and determined the thiocyanogen absorption of these specimens by the same procedure as followed with linseed oil. Thiocyanogen determinations of each sample were made with two and sometimes four solutions of approximately 0.19 N concentration. The results are shown in Tables III and IV.

The authors' thiocyanogen values of 98.1 for linoleic acid and of 167.4 for linolenic acid (168.0 calculated to a theoretical iodine number) are slightly higher than values of 96.3 and 167.3 by Kass and co-workers (*9, 10*), 97.3 and 167.6 by Riemenschneider, Swift, and Sando (*22*), and 96.6 and 166.3 by Matthews, Brode, and Brown (*19*). With the exception of linolenic acid, where the average excess of thiocyanogen reagent was 190 per cent the authors have made most of their determinations with an excess above 200 per cent. Kass and co-workers (*9, 10*) obtained their results when the excess reagent was 100 to 150 per cent; when the per cent of excess was raised to approximately 350 per cent with linoleic and to 250 per cent with linolenic, the thiocyanogen number increased about 3 points. The results of Riemenschneider, Swift, and Sando (*22*) fail to show as marked increases in the thiocyanogen number with increase in the excess of reagent as reported by Kass and co-workers. An inspection of the authors' data on the fat acids and esters fails to reveal any excess reagent effect. In nearly every case, however, the excess was over 190 per cent and the range was relatively small.

Matthews, Brode, and Brown (*19*) show, as predicted, that the thiocyanogen number of linolenic acid increases more with increase in amount of reagent than does that of linoleic acid. The

Table II. Reproducibility of Thiocyanogen Absorption Number of Linseed Oils

Oil 21		Oil 22		Oil 23	
Thiocyanogen solution No.	Thiocyanogen No.	Thiocyanogen solution No.	Thiocyanogen No.	Thiocyanogen solution No.	Thiocyanogen No.
15	121.1	23	114.5	37	117.0
16	119.0	24	115.2	39	115.9
17	122.1	24	115.7	42	114.9
17	120.8	25	113.7	44	116.6
18	121.4	26	114.0	45	117.4
19	121.1	29	113.5	46	116.4
19	121.2	30	115.6	47	115.4
..	...	31	113.6	..	...
..	...	32	114.9	..	...
..	...	33	116.4	..	...
..	...	34	115.3	..	...
..	...	35	113.2	..	...
..	...	36	114.1	..	...
..	...	37	114.8	..	...
..	...	39	116.1	..	...
Av.	121.0		114.7		116.2
Av. deviation from mean	±0.59		±0.85		±0.71
Maximum deviation from mean	2.0		1.7		1.3

Iodine No.: oil 21, 188.1; oil 22, 176.6; oil 23, 178.8.

Table III. Thiocyanogen Absorption of Linoleic Acid and Methyl Linoleate

Sample		Method of Preparation	Iodine No.	Thiocyanogen No.	Linoleic Acid Iodine No.	Linoleic Acid Thiocyanogen No.
Linoleic acid	A	Saponification of methyl ester. Distilled. Fraction 1	...	...	179.2	98.0
	B	Fraction 2 (above)	...	...	179.5	96.0
	C	Pyridine debromination. Not distilled	...	...	181.4	99.4
	D	Distilled (above) Fraction 1	...	...	181.2	98.0
	E	Distilled (above) Fraction 2	...	...	181.4	97.8
	F	Pyridine debromination. Distilled	...	...	181.2	98.6
	G	Saponification of methyl ester. Distilled	...	...	181.7	98.3
	H	Redistilled acid fractions	...	...	179.7	97.4
Methyl linoleate	A	H_2SO_4–MeOH debromination. Not distilled	172.0	93.3	180.6[a]	98.0[a]
	B	Distilled (above)	172.5	93.6	181.1	98.3
	C	H_2SO_4–MeOH debromination. Distilled. Fraction 1	172.6	94.1	181.2	98.8
	D	Fraction 2 (above)	172.2	93.6	180.8	98.3
	E	H_2SO_4–MeOH debromination. Distilled	172.4	94.1	181.0	98.8
	F	Redistilled ester fractions	171.4	93.5	180.0	98.2
		Av. thiocyanogen No.				98.1

[a] Methyl linoleate calculated as linoleic acid.

Table IV. Thiocyanogen Absorption of Linolenic Acid and Methyl Linolenate

Sample		Method of Preparation	Iodine No.	Thiocyanogen No.	Linolenic Acid Iodine No.	Linolenic Acid Thiocyanogen No.
Linolenic acid	A	Saponification of methyl ester. Distilled	...	...	271.2	167.7
	B	Pyridine debromination. Distilled. Fraction 1	...	...	271.6	167.0
	C	Fraction 2 (above)	...	...	272.3	167.6
	D	Saponification of methyl ester. Distilled. Fraction 1	...	...	271.7	168.1
	E	Fraction 2 (above)	...	...	272.5	168.3
	F	Pyridine debromination. Distilled. Fraction 1	...	...	272.5	168.8
	G	Fraction 2 (above)	...	...	272.5	168.5
Methyl linolenate	A	H_2SO_4–MeOH debromination. Redistilled. Fraction 1	259.9	158.0	273.0[a]	166.0[a]
	B	Fraction 2 (above)	258.8	157.3	271.8	165.2
	C	H_2SO_4–MeOH debromination. Distilled. Fraction 1	259.4	159.1	272.5	167.1
	D	Fraction 2 (above)	259.7	159.2	272.8	167.2
	E	H_2SO_4–MeOH debromination. Distilled. Fraction 1	259.9	159.4	273.0	167.4
	F	Fraction 2 (above)	259.3	159.8	272.4	167.8
	G	Redistilled ester fractions	259.1	159.1	272.2	167.1
		Av. thiocyanogen No.				167.4

[a] Methyl linolenate calculated as linolenic acid.

lower thiocyanogen values on the carefully purified samples prepared in Brown's laboratory (*19*) may be due to a lower reaction temperature (16° C.), and it is possible, though not likely, that the 10 per cent carbon tetrachloride lowered the absorption. Low thiocyanogen absorption numbers of linolenic acid samples stored for some time have been observed (*19*). The authors' thiocyanogen determinations were started the same day the linolenic samples were distilled. The iodine numbers were determined within a few hours after distillation. Most of the determinations on the linoleic samples were also made soon after preparation, but the iodine numbers of the linoleic samples, unlike the linolenic samples, dropped only slightly when stored several days in the refrigerator.

With few exceptions, the thiocyanogen numbers of the fat acids and esters (Tables III and IV) show excellent agreement in each group. The average thiocyanogen number of linolenic acid samples, 168.0, and of methyl linolenate samples, 166.8, a difference of 1.2 points, is difficult to explain. An average of these values must be chosen but it leaves some doubt as to the best choice of the empirical constant.

Norris, Kass, and Burr (*21*) favor the use of alcohol–hydrochloric acid (Rollett, *24*) for debromination and esterification in the preparation of esters of linolenic acid, but the authors have found Kimura's methyl alcohol–sulfuric acid reagent more satisfactory. Preparations of methyl linolenate by Rollett's method (*24*) had an iodine number 2 to 3 points lower than those shown in Table IV which were prepared by modifying Kimura's method. The thiocyanogen numbers were also lower, but when calculated to a theoretical iodine number, they were essentially the same as those shown in Table IV. After the hexabromostearic acid and zinc dust were ground together in a mortar, debromination in methyl alcohol proceeded smoothly. For the preparation of linolenic and linoleic acids, the authors find debromination in pyridine (*14*) much less tedious than saponification of the esters.

Composition of Linseed Oil

For several years samples of flaxseed grown at various locations throughout the United States and Canada have been sent to this laboratory. Samples with a range in iodine numbers from 127.8 to 202.8 have been collected. A linseed oil with an iodine number under 140 may seem surprising. Adverse climatic conditions, high temperature, and insufficient moisture while the seed ripens sometimes produce an oil in flaxseed with an iodine number this low. These conditions were not uncommon in 1936. Occasionally low iodine number oils have been produced in the Northwest Great Plains in other years.

The analytical results necessary for the calculation of the fat acid glycerides are shown in Table V.

The saturated acids were determined by the Bertram method (*3*), which gives higher values than the older lead-salt method. These are minimum values because the iodine number of the saturated acid specimens is essentially zero. No attempt was made to determine the different saturated acids. Small amounts of C_{20} and C_{24} acids (*25*) have been reported in linseed oil, but most of the saturated acids are stearic and palmitic. In order to calculate the fat acid composition as glycerides, the neutralization equivalent of several samples of saturated acids was determined. The average molecular weight of the saturated fat acids was 275.7. The neutralization equivalent of the saturated acids from several oils varied somewhat more than expected.

When substituting constants in simultaneous equations to secure expressions which give the percentages of the fat acids as glycerides, it is sometimes necessary to make corrections for the presence of the unsaponifiable matter and free fat acids in the oil. With linseed oil, however, corrections for these components do not appear to be necessary. The unsaponifiable portion has an iodine and thiocyanogen number not greatly different from that of the oil. Iodine values of 159.0, 164.5, 167.8, and 162.4 and thiocyanogen values of 106.2 and 116.5 have been obtained on individual samples. The combined unsaponifiable fraction of approximately 75 determinations, which accumulated over several months, gave an iodine number of 145.5 and a thiocyanogen

Table V. Analytical Results on Linseed Oils

Oil No.	Variety	Iodine No.	Thiocyanogen No.	Unsaponifiable %	Saturated Acids %
1	Unknown	127.8	89.1	1.34	15.37
2	Unknown	135.4	95.8	1.27	14.84
3	Unknown	146.2	99.7	1.10	12.00
4	Unknown	154.6	103.7	0.97	11.12
5	Unknown	164.8	107.2	0.94	11.19
6	Unknown	166.1	106.8	0.84	11.71
7	Unknown	177.0	113.5	0.82	11.12
8	Unknown	193.6	122.2	0.79	9.76
9	Unknown	200.0	124.4	0.84	8.52
10	Bison	162.8	107.8	0.88	10.70
11	Bison	162.9	107.8	0.91	13.12
12	Bison	164.5	110.8	0.98	9.92
13	Bison	176.8	114.6	0.85	8.46
14	Bison	181.1	115.6	0.83	8.96
15	Bison	185.6	119.5	0.86	8.96
16	Linota	176.9	115.1	1.10	8.33
17	Linota	199.2	125.0	0.80	6.94
18	Linota	202.8	127.2	0.91	6.93
19	Redwing	178.2	115.5	0.98	9.01
20	Redwing	195.1	122.9	0.95	7.96

Table VI. Composition of Linseed Oils

(Expressed in per cent of glyceride fraction)

Oil No.	Variety	Saturated Glycerides	Oleic Glycerides	Linoleic Glycerides	Linolenic Glycerides
		%	%	%	%
1	Unknown	16.3	40.5	22.7	20.5
2	Unknown	15.7	42.5	11.8	30.0
3	Unknown	12.7	37.1	19.3	30.9
4	Unknown	11.7	33.6	19.5	35.2
5	Unknown	11.8	26.4	22.2	39.6
6	Unknown	12.3	23.6	24.6	39.5
7	Unknown	11.7	21.4	18.5	48.4
8	Unknown	10.3	15.6	15.4	58.7
9	Unknown	9.0	11.9	19.4	59.7
10	Bison	11.3	30.3	18.1	40.3
11	Bison	13.8	28.6	13.9	43.7
12	Bison	10.5	33.8	11.7	44.0
13	Bison	8.9	25.3	19.2	46.5
14	Bison	9.4	21.1	21.2	48.3
15	Bison	9.4	22.0	14.4	54.2
16	Linota	8.8	26.1	17.9	47.2
17	Linota	7.3	15.1	19.0	58.6
18	Linota	7.3	14.2	16.7	61.8
19	Redwing	9.5	24.7	17.2	48.6
20	Redwing	8.4	16.1	18.4	57.1

of 90.4. Since these values do not differ greatly from those of the total oils (the IN/TN ratio is close to that of the oil), and the unsaponifiable fraction makes up approximately 1 per cent of the oils, it is unlikely that corrections for this fraction would amount to more than 0.2 point.

It follows that the equations recommended by Kaufmann and Baltes (*12*), which include an expression for the unsaponifiable matter, cannot be applied to linseed oil unless the iodine and thiocyanogen values of the unsaponifiable are determined and included in the expressions. If, on the other hand, the unsaponifiable portion is deleted in the calculations, the error due to its presence in the oil will be small, if not negligible.

The free fat acids, rarely higher than 1.5 per cent of the total, would if esterified reduce the iodine and thiocyanogen numbers slightly. Only in cases where the free fat acids were unusually high would an error of more than 0.1 per cent result by considering the free fat acids as glycerides in the calculations.

There is some question as to whether or not oleic acid adds thiocyanogen quantitatively. Most results (*22*) indicate a thiocyanogen number 0.4 to 0.5 point lower than the theoretical, but oleic acid is difficult to obtain completely free of saturated and linoleic acids. The presence of these would result in low thiocyanogen values. Matthews, Brode, and Brown (*19*) state that their pure samples of oleic acid absorb thiocyanogen quantitatively, and Wheeler, Riemenschneider, and Sando (*26*) obtained nearly quantitative results on triolein. The authors, therefore, favor the theoretical value of 89.9.

When simultaneous equations employing thiocyanogen absorption numbers of 89.9 for oleic acid, 98.1 for linoleic acid (Table III), and 168.0 for linolenic acid (Table IV), and theoretical iodine numbers of 89.9, 181.0, and 273.5, are solved, the following expressions are obtained:

$$\begin{aligned} \%\ \text{oleic acid} &= 1.649\,TN - 1.246\,IN - 0.638\,S + 63.8 \\ \%\ \text{linoleic acid} &= -3.269\,TN + 1.391\,IN - 1.689\,S + 168.9 \\ \%\ \text{linolenic acid} &= 1.624\,TN - 0.146\,IN + 1.329\,S - 132.9 \end{aligned}$$

where TN = thiocyanogen absorption number, IN = iodine absorption number, and S = per cent saturated acids.

By a similar process, with the above thiocyanogen and iodine numbers converted to values corresponding to glycerides, the expressions become:

$$\begin{aligned} \%\ \text{oleic glyceride} &= 1.724\,TN - 1.303\,IN - 0.638\,S + 63.8 \\ \%\ \text{linoleic glyceride} &= -3.426\,TN + 1.457\,IN - 1.693\,S + 169.3 \\ \%\ \text{linolenic glyceride} &= 1.701\,TN - 0.154\,IN + 1\,330\,S - 133.0 \end{aligned}$$

The composition of the linseed oils listed in Table V, expressed as glycerides, is shown in Table VI.

With the fat acid composition calculated as per cent of glycerides of the total glyceride fraction, the results should be directly comparable to results obtained by analysis of the fat acids obtained by saponification. A comparison of the percentages of fat acids calculated from analysis of the fat acids with those calculated from analysis of the oil should indicate the precision of the authors' methods of analysis. A few results on fat acids obtained by saponification of some of the linseed oils are shown in Table VII.

For the most part, the results on the fat acids agree well with those on the oils. When the numerous sources of errors in the procedures for fat acid analysis are considered, these results seem satisfactory. Analysis of synthetic mixtures of methyl esters of oleic, linoleic, and linolenic acids by Riemenschneider, Swift, and Sando (*22*) and of the acids by Matthews Brode, and Brown (*19*) gave results similar to those in Table VII.

Analyses of fat acids obtained by saponification have certain advantages, but the authors prefer to make determinations on the oil itself when it is as unsaturated as linseed oil. Many fat acid samples prepared by methods which require prolonged heating in strongly alkaline solutions (*4*) gave percentages of fat acids much different from the oils. The iodine numbers were invariably lower than should have resulted from the parent oils. Without exception the linolenic acid content of these fat acids was lower than that of the oils. Linseed oil is fairly stable, but the manipulations necessary for the preparation of fat acids may produce changes. A shift of ethylenic bonds, polymerization, and oxidation may occur. When the fat acid samples were prepared by a quick saponification the fat acid composition was reasonably close to that of the oils (Table VII).

The composition of linseed oil, when calculated from empirical constants, shows quite a different picture from the composition calculated from the older theoretical constants. The chief differences are that the oleic and linolenic acid content is considerably higher, and the linoleic acid content considerably lower, than formerly believed. Linoleic acid values higher than 40 per cent have often been reported (*8*). When the composition of the linseed oils shown in Table V is recalculated by employing theoretical constants proposed by Kaufmann and Baltes (*12*) the values are much different from those shown in Table VI. One sample gave an oleic acid content of less than 4 per cent.

The composition of the oils in Table VI agrees well with that published by Rose and Jamieson (*25*), who, although they did not determine constants for the thiocyanogen absorptions, made their calculations using constants reported by others for 0.1 N thiocyanogen solutions. Hilditch and Murti (*7*) reported on the composition of three linseed oils by recalculation of previously published results using their thiocyanogen numbers. Unless the conditions under which the former determinations were carried out are known, and the thiocyanogen constants determined under similar conditions, this is a bold procedure. Unfortunately, the data in most cases are insufficient to permit a recalculation of the results already published. When any of the known factors which affect the thiocyanogen absorption are changed, other predetermined empirical values are necessary. The authors agree with Rose and Jamieson (*25*) that "in the majority of cases where thiocyanogen values are given in the literature for linseed and other oils of the same type containing notable quantities of linolenic acid, there is apparently no way of knowing the empirical values which should be used for recalculating the percentages of the individual unsaturated acids in these oils."

Experimental

Determination of Iodine Number. Wijs 1-hour absorption, 100 to 150 per cent excess.

Determination of Thiocyanogen Number. Fifty grams of the lead thiocyanate (*1*) were suspended in 500 cc. of the acetic acid reagent (glacial acetic refluxed 3 hours with 10 per cent acetic anhydride) and approximately 450 cc. of the acid

containing 17 grams of bromine were added in small portions. After the lead bromide had been filtered out the clear thiocyanogen solution was titrated and the concentration adjusted to 0.18 to 0.19 *N* (recently 0.185 to 0.190 *N*) by addition of acetic acid reagent.

Determinations were carried out in 250-cc. glass-stoppered flasks held at 20 ± 1° C. for 24 hours. Titration has been described (*1, 9*).

Determination of Saturated Fat Acids and Unsaponifiable Matter. The unsaponifiable matter was determined by the modified Kerr-Sorber method (*8*) and the saturated fat acids by the modified Bertram (*3*) method as given by Jamieson (*8*) with the following changes. (*a*) The sulfuric acid and bisulfite were added alternately and in small portions to destroy the excess permanganate and manganese oxides, and to reduce frothing. A few drops of capryl alcohol were added to aid in reducing the frothing. (*b*) The extraction of the fat acids, before and after the precipitation of the magnesium soaps, was made with smaller quantities of petroleum ether, first with 150 cc., then decreasing to about 75 cc. for the third and fourth extractions.

Linseed Oil Samples. The oil samples were pressed from finely ground flaxseed warmed to about 60° C.

Table VII. Comparison of Results by Analyses of Oils and Fat Acids

Oil No.	Saturated Oil[a]	Saturated Fat acids	Oleic Oil[a]	Oleic Fat acids	Linoleic Oil[a]	Linoleic Fat acids	Linolenic Oil[a]	Linolenic Fat acids
	%	%	%	%	%	%	%	%
7	11.7	11.9	21.4	23.8	18.5	14.7	48.4	49.7
8	10.3	10.0	15.6	17.0	15.4	13.8	58.7	59.1
10	11.3	11.1	30.3	30.3	18.1	19.2	40.3	39.4
11	13.8	14.2	28.6	27.8	13.9	13.1	43.7	44.8
12	10.5	10.8	33.8	34.7	11.7	10.2	44.0	44.2
13	8.9	9.4	25.3	25.7	19.2	17.2	46.5	47.6
15	9.4	9.6	22.0	23.5	14.4	15.0	54.2	51.8
16	8.8	8.4	26.1	27.7	17.9	17.6	47.2	46.3
19	9.5	9.7	24.7	26.0	17.2	13.0	48.6	51.2
20	8.4	8.7	16.1	17.9	18.4	15.6	57.1	57.8

[a] Results on oils expressed as glycerides.

Quick Saponification and Separation of Fat Acids. Fat acids shown in Table VII were prepared by the following method: To 25 grams of linseed oil in a 2-liter Erlenmeyer flask, approximately 25 ml. of 95 per cent ethyl alcohol were added. The flask was then placed in a boiling water bath and when the temperature of the oil solution reached that of the boiling water bath, 5 ml. of 50 per cent sodium hydroxide (specific gravity 1.53) were added slowly from a pipet. Saponification was complete in 3 to 5 minutes. The soap solution was then removed from the water bath and diluted with approximately 800 ml. of water, and the unsaponifiable material was extracted by ether. Dilute sulfuric acid (1 + 4) was added to liberate the fat acids. After the fat acids were washed with several portions of water, they were dissolved in petroleum ether and dried with anhydrous sodium sulfate and the solvent was removed on the steam bath at reduced pressure. During all the steps following saponification a stream of nitrogen was passed through the solutions.

Preparation of Bromides of Linoleic and Linolenic Acids. Fat acids of linseed oil and sunflower oils (*4*) dissolved in dry ethyl ether and in petroleum ether were cooled to approximately −20.56° C. (−5° F.) by placing outside on a cold day, and brominated.

The linolenic hexabromide was filtered out, washed copiously with ether, dissolved in the minimum amount of hot (about 80° C.) 1-4,dioxane (*18*), filtered by the aid of a steam-jacketed funnel, and then allowed to cool. When crystallization began, an equal volume of petroleum ether was added. The crystalline hexabromide was filtered after standing at 0° C. overnight and the product washed well with petroleum ether. The hexabromide was recrystallized two more times in this manner and once treated with decolorizing carbon before filtration of the solution in hot dioxane. After a final crystallization from boiling xylene, the melting point was 182.5° C. (melting point tube inserted at 175° C.).

The linoleic tetrabromide was first washed with petroleum ether, then crystallized four times by the addition of 3 volumes of petroleum ether to a saturated solution of tetrabromide. Before the third recrystallization the ethyl ether solution was treated with decolorizing carbon, warmed to a boil, and filtered. The product was less soluble in ethyl ether than reported by McCutcheon (*17*). Melting point = 115–115.5° C.

Preparation of Linoleic Acid, Methyl Linoleate, Linolenic Acid, and Methyl Linolenate. All debrominations, solvent removals, and distillations were carried out in all-glass apparatus with ground-glass connections and in an atmosphere of nitrogen. The methyl alcohol used was distilled from magnesium methoxide. The final products were taken up in petroleum ether (Skellysolve F, boiling point 30° to 60° C.). After the solutions had been dried with anhydrous sodium sulfate the petroleum ether was removed by the use of a water pump and the products were distilled. A vacuum receiver of the type described by Noonan (*20*) permitted a separation into fractions without disturbing the distillation. The usual practice was to collect a sample in a receiving flask until the boiling point became constant (fraction 1), then turn the stopcock of the receiver to separate the constant-boiling fraction (fraction 2).

Methyl Linolenate. Forty grams of hexabromostearic acid and 40 grams of zinc dust were ground together in a mortar and suspended in 90 ml. of methyl alcohol. After refluxing 1 hour, a solution of 15 ml. of concentrated sulfuric acid in 100 ml. of methyl alcohol (*15*) was slowly added. The solution was refluxed 1 hour and the ester separated (*22*). The petroleum ether solution was washed with dilute sodium carbonate. One product distilled at 109° C. at 0.018 mm., another at 114° C. at 0.030 mm.

Linolenic Acid. Samples A, D, and E were prepared by saponification of methyl linolenate in the cold in a completely filled flask as suggested by Kass, Loeb, Norris, and Burr (*9*). After most of the alcohol was removed at reduced pressure, the solution was diluted with water and extracted with petroleum ether to remove any unsaponified ester before liberation of the acid.

Samples B, C, F, and G were prepared by pyridine debromination (*14*). To 40 grams of hexabromostearic acid and 40 grams of zinc ground together in a mortar, 120 ml. of freshly distilled pyridine were added. As the reaction was rather violent, the flask was cooled with ice water. After the initial exothermic reaction subsided, the flask was immersed in a boiling water bath for 15 minutes. While still warm the semisolid mass was transferred to a separatory funnel with the aid of approximately 100 ml. of concentrated hydrochloric acid in 400 ml. of water, and petroleum ether. The lumps of zinc salt were broken up with a glass rod and shaken up with petroleum ether. The pyridine was extracted with water. One product distilled at 126° C. at 0.05 mm., another at 137° C. at 0.07 mm.

Methyl Linoleate was prepared by same method as methyl linolenate except that 40 grams of tetrabromide, 30 grams of zinc, and 110 ml. of methyl alcohol were used to debrominate, and a solution of 8 ml. of concentrated sulfuric acid in 70 ml. of methyl alcohol was used to complete esterification. Products distilled at 121° C. at 0.04 mm. and at 147° C. at 0.32 mm.

Linoleic Acid. Methyl linoleate was saponified in the same manner as methyl linolenate. Tetrabromostearic acid was debrominated with zinc in pyridine as described for hexabromostearic acid. Fractions were collected at 129° C. at 0.018 mm. and at 133° C. at 0.070 mm.

After preparation, the acid and ester fractions were placed in vials, most of the air was displaced by nitrogen, and the stoppered vials were stored in the refrigerator.

Summary

The thiocyanogen absorption of linseed oil has been studied to show the importance of controlling the concentration of reagent, the absorption time, and the excess of reagent. Samples of linoleic acid, methyl linoleate, linolenic acid, and methyl linolenate were prepared to determine the empirical thiocyanogen absorption numbers by the authors' method. The average thiocyanogen absorption values for linoleic, 98.1, and for linolenic, 168.0, were then used in equations to obtain expressions to calculate the composition of linseed oils. Linseed oils with iodine numbers from 127.8 to 202.8 were analyzed. A comparison of the results calculated from analyses of the fat acids, obtained by saponification, with those calculated from analyses of the original oils, showed fair agreement. The range of the saturated glycerides was from 7.3 to 16.3 per cent, of the oleic glycerides from 11.9 to 42.5 per cent, of the linoleic glycerides from 11.7 to 24.5 per cent, and of the linolenic glycerides from 20.5 to 61.8 per cent. Linseed oil contains more linolenic acid, more oleic acid, and less linoleic acid than formerly supposed.

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Cryoscopic Analysis of Styrene, Indene, and Dicyclopentadiene

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SMALL quantities of certain impurities in monomeric styrene, indene, and dicyclopentadiene markedly affect the properties of polymers produced therefrom. Limitations of analytical methods for the detection of small quantities of impurities based on bromine absorption or on the accurate measurement of several physical properties are well known to investigators in this field.

A method for determining the amount of impurity in hydrocarbons from freezing and melting curves is described by Mair, Glasgow, and Rossini (*1*) but is not designed for control work where speed is essential.

It was believed that an analytical procedure based on the depression of the freezing point of the hydrocarbon would provide a precise and sensitive method, dependent on the relative number of molecules of hydrocarbon impurities present and practically independent of the type of impurities.

The development of the procedure entailed: (1) the preparation of "100 per cent" styrene, indene, and dicyclopentadiene, respectively, and (2) the design of a suitable apparatus for measuring freezing points, and the determination of the molal dispersions, K_f, of the compounds stated in (1).

Purification of Light Oil Hydrocarbons

The light oil hydrocarbons were purified by successive recrystallizations. The liquid phase was separated from the solid by means of a centrifuge. The centrifuge cakes were not washed. Advantage of equilibrium melting was taken during centrifuging. The criterion of purity consisted of identity of freezing points of the solid and liquid phases of the final crystallization. The hydrocarbons thus processed were assumed to be pure. The purified compounds had the following physical properties.

Compound	Freezing Point, ° C. (Cor.)	Refractive Index, n_D^{20}
Styrene	−30.60	1.5469
Indene	− 1.50	1.5764
Dicyclopentadiene	33.6	....

Measurement of Freezing Points and Molal Depressions

Styrene. The cell in which the freezing points were determined was a glass tube 38 × 1.6 cm. (inside diameter) surrounded by a tube 39 × 2.5 cm. (inside diameter) which provided an air bath. A specially constructed mercury thermometer, 37.5 cm. long, graduated directly to 0.01° C. in the range of −30° to −38° C. was used to measure temperatures. The thermometer was calibrated by the National Bureau of Standards.

A 15-cc. sample, which immersed the thermometer to the −37° mark, was found to be sufficiently large. The freezing cell and air bath were immersed to the −35° mark on the thermometer scale in a carbon dioxide–acetone bath contained in an unsilvered quart Dewar vessel. The bath was maintained at a temperature approximately 5° C. lower than the freezing point of the solution under examination. The solution, the freezing point of which was being determined, was stirred mechanically with a 12-gage Nichrome wire, provided with two loops surrounding the thermometer below the solution level. The highest temperature observed after crystallization began was taken as the freezing points.

Solutions of varying composition were prepared from purified styrene and purified *m*-xylene. The freezing points of the solutions were determined and the molal depression constant was calculated for each ΔT between consecutive solutions. Two observers independently checked each freezing point with the deviation shown, along with the complete data, in Table I.

Indene and Dicyclopentadiene. Purified indene and purified dicyclopentadiene, respectively, were diluted with *p*-

Table I. Freezing Data of Styrene-Xylene Solutions

Styrene *Mole %*	Freezing Point °*C.* (*cor.*)	Super-cooled °*C.*	Maximum Deviation between Observers °*C.*	*Kf*
100	−30.60	0.5	0.00	..
99.77	−30.69	0.6	0.00	4.9
99.58	−30.78	0.5	0.00	5.3
99.22	−30.95	0.4	0.00	4.9
99.08	−31.01	0.6	0.00	4.9
98.15	−31.44	0.4	0.00	5.1
96.61	−32.15	0.6	0.01	4.9
95.30	−32.76	0.7	0.01	5.0
			Av.	4.95

Table II. Freezing Data

Indene Mole %	Freezing Point °C. (cor.)	Supercooled °C.	Kf
	Indene–p-Xylene Solutions		
100	−1.50	0.3	..
99.34	−1.91	0.4	6.86
98.85	−2.20	0.3	6.90
98.47	−2.46	..	6.88
97.93	−2.78	0.4	6.88
		Av.	6.89
Dicyclopentadiene Mole %	**Freezing Point °C.**		
	Dicyclopentadiene–p-Xylene Solutions		
100	33.6	0.1	..
99.63	32.25	0.1	55.1
99.25	31.1	0.1	48.2
98.88	29.5	0.1	51.0
98.38	27.1	0.1	50.0
96.79	21.4	0.1	49.3
		Av.	50.7

xylene (freezing point 13.3° C.) and the freezing points were measured by means of a thermometer, graduated directly to 0.01° C., calibrated by the National Bureau of Standards. The freezing point technique was in general identical with that described under styrene. Results are summarized in Table II.

Discussion

The data presented indicate a precise analytical method for the detection of small quantities of impurities in the hydrocarbon examined. Compounds higher in molecular weight than xylene produce a proportionally lower freezing point depression per unit weight, and impurities of lower molecular weight produce a depression proportionally higher. High molecular weight polymers in small amounts would not be detected by this method. However, impurities of boiling points diverging appreciably from the boiling point of the light oil hydrocarbons in question can be separated by fractional distillation. Chemically reactive compounds, such as organic acids which produce an abnormally high K_f, should be determined and removed by chemical means. The cryoscopic procedure is used in research and control work only between concentrations of 92 to 100 per cent.

The true freezing points of the solutions of purified compounds and xylene are slightly higher than those herein reported, for reasons discussed by Mair, Glasgow, and Rossini (*1*).

The high K_f and convenient freezing point of dicyclopentadiene suggest a possible use in the determination of molecular weights as a substitute for camphor. The reactivity of the compound, especially with respect to oxidation, would necessitate some precautions not required in the case of camphor.

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Determination of Sulfur Dioxide in Beer

A Modification of Monier-Williams Method

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During the determination of sulfur dioxide in beer by the Monier-Williams method (*1*), it was noticed that a precipitate of barium sulfate was formed in the oxidizing medium (3 per cent hydrogen peroxide). This was found to be due to an excess of barium hydroxide added in removing the sulfate ion from the peroxide by the regular procedure. The presence of this precipitate indicated that any sulfur dioxide determinations run using this hydrogen peroxide were in error by the amount of sulfate precipitated, as sulfate in this form is not titratable.

An attempt was made to improve the accuracy of the procedure as well as increase the rapidity with which the hydrogen peroxide could be prepared by eliminating the effect of the sulfate by another method not involving the use of barium hydroxide, as it is difficult to avoid adding an excess of this reagent. This was accomplished by neutralizing the acid in the peroxide with 0.1 *N* sodium hydroxide to pH 4.0, using a glass electrode (the neutralization could be made to the bromophenol blue end point). pH 4.0 is the neutral point in the titration of the sulfuric acid by this method.

The sulfuric acid in the peroxide is not removed as it is when barium hydroxide is used, but the effect of any acid present in the peroxide is eliminated by neutralization, thus avoiding any error in the volumetric result due to sulfuric acid in the peroxide.

Should it be necessary to check the volumetric result gravimetrically, a blank on the hydrogen peroxide must be determined by precipitating the sulfate present with barium chloride and this blank deducted from the total barium sulfate.

Experimental

Excess Barium Ion in Hydrogen Peroxide Neutralized with Barium Hydroxide. Two separate portions of 3 per cent hydrogen peroxide were prepared in regular manner by diluting Superoxol (Merck 30 per cent) and precipitating the sulfate present with 10 per cent barium hydroxide solution to the bromophenol blue end point. The precipitate of barium sulfate was allowed to settle for 5 days at 5° C., when the solution was filtered, standardized with 0.1 *N* potassium permanganate, and diluted to exactly 3 per cent.

Gravimetric blanks (Table I) were determined on the peroxide solutions to estimate the excess barium ion and the error due to its presence. This was done by adding 1 ml. of concentrated sulfuric acid to 20 ml. of the peroxide, and calculating the weight of

Table I. Determination of Blanks

(20-ml. sample)

Sample	SO_2 Equivalent to Excess Barium Ion *P. p. m.*
1	0.84
2	1.02
Av.	0.93

barium sulfate thus precipitated to the equivalent amount of sulfur dioxide. The presence of excess barium ion results in a negative error and the blank must be added to any determinations of sulfur dioxide run when using peroxide prepared as directed above, as the first portion of sulfuric acid formed by the oxidation of sulfur dioxide will be precipitated and thus cannot be titrated, giving a low result.

Preparation of Hydrogen Peroxide (Barium-Free). Twelve milliliters of Superoxol (Merck 30 per cent) were diluted to 100 ml. with distilled water, and then neutralized with 0.1 *N* sodium hydroxide to pH 4.0, using a glass electrode. About 4 ml. of the base were required. The strength was then determined by permanganate titration and the neutral solution diluted to exactly 3 per cent. Experience has shown, however, that this standardization is necessary only when the Superoxol is of old stock and considerably too weak, as the peroxide solution required need only be approximately 3 per cent in strength.

Titration blanks were determined on this solution, using a 20-ml. sample, by running a regular sulfur dioxide determination, replacing the usual beer sample with 300 ml. of distilled water. The peroxide was then washed into a 100-ml. beaker and the pH determined. In all cases the pH was 4.0. Had the pH been either higher or lower than 4.0, the solution would have been titrated to that pH using either 0.01 *N* sodium hydroxide or 0.01 *N* sulfuric acid, and this titration subtracted or added to the titration for each regular sulfur dioxide determination, depending on the standard solution used.

Sulfur Dioxide Determinations (Table II). Volumetric determinations were run by the Monier-Williams method (*1*) using both peroxide neutralized with barium hydroxide to the bromophenol blue end point and peroxide neutralized to pH 4.0 (glass electrode) with 0.1 *N* sodium hydroxide. The titrations with 0.01 *N* sodium hydroxide to pH 4.0 were made using a glass electrode.

The procedure as outlined in the Monier-Williams method (*1*) was used in gravimetric determinations, with the exception that the barium sulfate precipitate was digested by keeping the solution at incipient boiling for one hour, then holding it on the steam bath for one hour, and allowing it to stand overnight.

Table II. Gravimetric-Volumetric Checks

(Hydrogen peroxide neutralized with barium hydroxide)

Sample No.		SO_2 Volumetric Uncorrected	SO_2 Volumetric Corrected[a]	SO_2 Gravimetric	SO_2 Difference Uncorrected	SO_2 Difference Corrected
		P. p. m.	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*
1		11.2	12.0	12.35	1.15	0.35
1		10.9	11.7	12.10	1.20	0.40
	Av.	11.1	11.9	12.20	1.10	0.30
2		13.4	14.2	14.35	0.95	0.15
2		13.8	14.6	14.50	0.70	−0.10
	Av.	13.6	14.4	14.43	0.83	0.03

[a] Blank compensating for excess barium in peroxide added.

Discussion of Results

For convenience, the blanks on the peroxide solutions (Table I) were calculated to parts per million of sulfur dioxide in determinations using 300 ml. of beer, rather than to milligrams of sulfur dioxide.

Correcting the results of volumetric determinations of sulfur dioxide (Table II) in beer for the error due to excess barium ion in the peroxide brings them into closer agreement with the results determined gravimetrically.

In determinations of sulfur dioxide in beer, the use of peroxide neutralized with sodium hydroxide gives results approximately 1 part per million higher than those in which the peroxide was neutralized with barium hydroxide (Table III).

It can be seen from Tables II and III that several separate determinations of sulfur dioxide in the same beer sample can be checked within 0.5 p. p. m.

Summary

The proposed method of preparing hydrogen peroxide by neutralizing with 0.1 *N* sodium hydroxide to a pH of 4.0 is very rapid, thus making the determination available at any time; while preparation by the regular method requires a 5-day wait and a filtration before the peroxide can be used.

The presence of excess barium ion in the hydrogen peroxide prepared by neutralization with barium hydroxide to the bromophenol blue end point, as done in the regular procedure, causes an error approximately equivalent to 1 part per million of sulfur dioxide when a 300-ml. sample of the beer is used.

Hydrogen peroxide prepared by neutralizing with 0.1 *N* sodium hydrogen hydroxide to a pH of 4.0 eliminates the above error without otherwise impairing the accuracy of the procedure.

Gravimetric checks of the procedure may still be made if desired, providing a blank is run to determine the quantity of sulfuric acid present in the peroxide solution used.

The bromophenol blue indicator gives a satisfactory end point when 0.1 *N* sodium hydroxide is used. However, the end point is not sharp when 0.01 *N* sodium hydroxide is used, and the use of the potentiometric method of titration eliminates the uncertainty of the final end point with the lower concentration acid.

The above procedure is not restricted to beer alone, but may be used for determination of sulfur dioxide in many other materials such as malt, barley, corn sirup, wine, etc.

Table III. Reproducibility and Comparative Results

[Hydrogen (1) neutralized with sodium hydroxide, (2) neutralized with barium hydroxide]

Sample No.	H_2O_2 Neutralization	SO_2	Average	Average Diff.
		P. p. m.	*P. p. m.*	*P. p. m.*
3	1	5.6	5.6	0.50
3	2	4.8, 5.5	5.1	
4	1	15.8, 16.0	15.9	1.3
4	2	14.6	14.6	
5	1	8.8, 8.7	...	...

Acknowledgment

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Perchloric Acid Method for Determination of Silicon in Ferrosilicon

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IN THE tentative A. S. T. M. method for the determination of silicon in ferrosilicon (*1*), a large excess of sodium peroxide is used to reduce the violence of the reaction. The large quantity of sodium chloride subsequently formed in the solution increases the solubility of silica and produces low results (*5*). Two dehydrations with hydrochloric acid are needed to separate the silica quantitatively. This requires a great deal of time, particularly because of slow evaporations. Perchloric acid may be used as a dehydrating agent for silicon-rich ferrosilicon, if the samples are first decomposed (*3*, *7*).

A rapid empirical procedure has been evolved from these methods for the analysis of simple ferrosilicon samples containing approximately 25 to 75 per cent of silicon. Precise results may be obtained in about 5 hours with a minimum amount of manipulation.

TABLE I. DETERMINATION OF CORRECTION FACTOR

Sample[a]	Si Present	Si Found[b]	Error	Ratio of Si Present: Found
	%	%	%	
1	26.83	26.36	0.47	1.018
2	50.0	49.28	0.72	1.015
3	63.16	62.15	1.01	1.016
4	75.60	74.39	1.21	1.016
5	96.80	96.00[c]	0.80	1.008[d]
		Average correction factor		1.016

[a] Analyzed samples 1 and 3 were obtained from the Union Carbide and Carbon Research Laboratories, Niagara Falls, N. Y.; samples 2, 4, and 5 (refined silicon) are Bureau of Standards samples 59, 58, and 57, respectively.
[b] Each value represents average of duplicate determinations.
[c] One-fourth factor weight of sample was used.
[d] Omitted from average.

Procedure

A fusion mixture is prepared by grinding 10 grams of anhydrous sodium perchlorate (*8*), 20 grams of anhydrous sodium carbonate, and 70 grams of sodium peroxide in a mortar heated to about 100° C. The mixture is dried for 1 hour at 110° C. and kept in a desiccator.

About 1 gram of the fusion mixture is melted in a 16-ml. nickel crucible, which is slowly rotated as the melt sets to produce a uniform lining about 0.47 cm. ($^3/_{16}$ inch) in height. A 0.2336-gram sample (one-half factor weight) of ferrosilicon, ground to 200-mesh and dried for 1 hour at 110° C., is carefully mixed with 3 grams of the fusion mixture in the lined crucible with an iron rod. Particles adhering to the rod and to the sides of the crucible are brushed down, and the mixture is covered with about 1 gram of the fusion mixture.

The mixture is fused by revolving the crucible around the outer edge of a low burner flame. At the beginning of the reaction, which is detected by a characteristic sound, the crucible is set aside for about 3 minutes, after which it is heated at 700° C. for 15 minutes. The crucible when slightly cooled is inverted over the opening of a Parr bomb plate which is placed over a 200-ml. platinum dish. The melt may be removed easily by tapping the bottom of the crucible, although it is sometimes necessary to heat the crucible for a moment with a burner.

The platinum dish is partly covered with a watch glass and 60 to 75 ml. of water, followed by 12.5 ml. of 12 *N* hydrochloric acid, are added to the melt. The crucible is rinsed with about 10 ml. of water, filled with 3 *N* hydrochloric acid which is left until the residue has completely dissolved, and again rinsed with water. All the washings are to be added to the contents of the dish. The analysis is rejected if any particles of unreacted ferrosilicon are observed.

The acidic solution is transferred to a 400-ml. Pyrex beaker, and 50 ml. of 70 per cent perchloric acid are added. The mixture is concentrated to heavy white fumes over a hot plate set at 85° to 135° C.; the evaporation may be greatly hastened by the use of a drying lamp. The beaker, covered with a Pyrex watch glass, is then heated above 200° C. for 20 minutes, during which the acid should boil freely and reflux along the sides of the beaker.

The mixture is cooled to about 100° C., diluted with 200 ml. of boiling water, and digested over a steam bath for 15 minutes. The precipitate is washed three times with hot 1 per cent hydrochloric acid, using a total of 125 ml., transferred to a 41-H Whatman filter paper (*2*), and washed six times with hot water.

The precipitate is ignited in a platinum crucible at 1050° C. for 30 minutes, after the paper has been charred and burned over a low flame. The crucible is cooled in a desiccator and weighed. The silica is moistened with concentrated sulfuric acid, and dissolved in 15 to 20 ml. of pure 48 per cent hydrofluoric acid. The solution is evaporated to dryness, and the crucible weighed again.

The per cent of silicon in the sample is found by multiplying the weight of silica (loss by hydrofluoric acid treatment) by 203.2, which is the product of 200 and a correction factor of 1.016 (Table I).

Discussion

Practically all the sources of error in the procedure are negative, as Hawley (*4*) and others (*6*) have pointed out in connection with similar determinations. The most significant error results from the loss of a definite amount of silicic acid which escapes dehydration, but conditions may be controlled in such a way that the total loss of silica is nearly proportional to the per cent of silica in the sample. A correction factor may therefore be introduced to avoid the necessity of a second dehydration.

It is evident from the calculations summarized in Table I that highly precise results may be obtained for ferrosilicons containing approximately 25 to 75 per cent of silicon by applying the correction factor of 1.016. The factor should be checked occasionally by the analysis of a ferrosilicon of known silicon content, particularly if the procedure is changed significantly. For instance, the method may be applied to refined silicon by reducing the size of the sample to one-fourth factor weight if a correction factor of 1.008 is used.

Summary

In a simplified method for the determination of silicon in a comparatively wide range of ferrosilicon samples, the sample is fused with a mixture of sodium peroxide, sodium carbonate, and sodium perchlorate. The melt is then decomposed with water and hydrochloric acid, and the resulting silicic acid is dehydrated with perchloric acid. A correction factor is introduced to eliminate the necessity for a second dehydration.

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Photoelectric Fluorimeter

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THE alternating current–operated fluorimeter shown in Figure 1 attains its sensitivity by the use of vacuum photocells whose current is amplified by an electronically stabilized feedback amplifier of conventional design, similar to that described by Krebs, Perkins, Tytell, and Kersten (*1*).

An ultraviolet lamp housed in a cylinder open at both ends (*C*, Figure 1) provides the light which passes through four 0.47 × 2.5 cm. ($^3/_{16}$ × 1 inch) vertical slits and emerges in the direction of the white arrow. The cells for holding the liquid whose fluorescence is to be determined are supported on two platforms of an "elevator". One cell is shown in position at *G* and the platform for the other is at *E*. Part of the fluorescence from the liquid enters two photocells contained in housings (one shown at *F* and the other opposite it) after it passes through filters held in position at *D*. One of the two windows through which the fluorescent light passes is shown at *H* and the other is opposite it. The filters may be selected to transmit a part of the fluorescent light without transmitting too much of the light diverted toward the photocells from the ultraviolet beam by the liquid. A liquid which does not fluoresce will then have a reading of almost zero on the meter.

After the fluorimeter is wired, *R*5 is adjusted until the voltage across the filament leads of *T*9 is 12.6. This is the only permanent adjustment which need be made. To measure fluorescence with the instrument, *S*2 is closed; after about a minute *S*1 is closed, the ultraviolet lamp is turned on, and the whole apparatus is allowed to warm up for 15 minutes. The liquid is then poured into cell *G* and the solvent into a similar cell which is placed on the platform at *E*. The door is closed and the meter brought to zero by turning rheostats *R*12 and *R*9 (one is for coarse and the other for fine adjustment). Next, cell *G* is raised to a position formerly occupied by the one on platform *E* by means of rod *A* which slides without rotating in tube *B*. The reading of the meter will then indicate a number proportional to the fluorescent light from the sample. The cells are raised and lowered several times to assure the operator that the circuit and the light have remained in a stable state during the several seconds needed to take the readings. One may also use only one cell, in which case the meter is set to zero when this cell is in position *G*.

The sensitivity of the instrument depends on the size of the resistor, *R*10. Figure 3 shows a calibration curve made when *R*10 had a value of 1000 megohms. The ultraviolet lamp was found to be exceedingly stable against voltage fluctuations, probably because its gaseous discharge acted as a negative, while its transformer acted as a positive resistance, resulting in a combination which produced a fixed light output independent of small line voltage variations.

The following parts are needed: *R*1, 5000 ohms, 0.5 watt; *R*2, 7500 ohms, 10 watts; *R*3, 100,000 ohms, 2 watts; *R*4, 5000 ohms, 10 watts; *R*5, 100,000-ohm potentiometer; *R*6, 100,000 ohms, 2 watts; *R*7, 1000 ohms, 25 watts, adjusted so that 0.15 ampere flows through it; *R*8, 2500 ohms, 2 watts; *R*9, 1000-ohm potentiometer; *R*10, 1000 megohms; *R*11, 150 ohms, 0.5 watt; *R*12, 1000-ohm potentiometer; *R*13, 50,000 ohms, 2 watts; *R*14, 3 megohms, 2 watts; *R*15, 120,000 ohms, 2 watts; *R*16, 20,000 ohms, 0.5 watt; *C*1 16-mfd., 450-volt, electrolytic condenser; *B*1, 22.5-volt B-battery (this will need to be changed about once per year); M, 0-200 microammeter; *S*1, double-pole single-throw toggle switch; *S*2, double-pole single-throw toggle switch; *TR*, Thordarson T13R09 transformer; *CH*1, Thordarson

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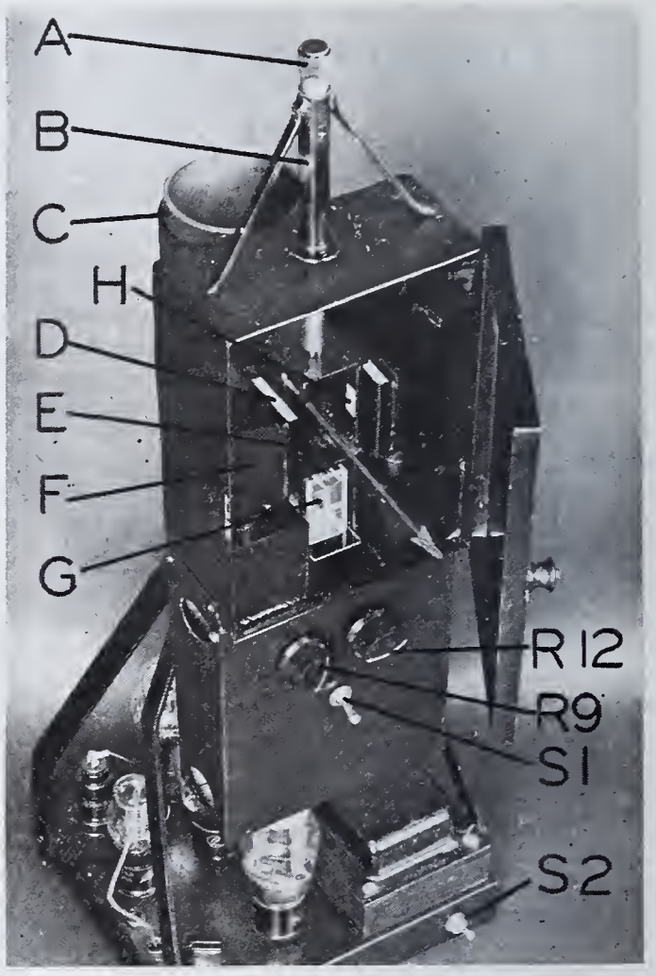

FIGURE 1. FLUORIMETER

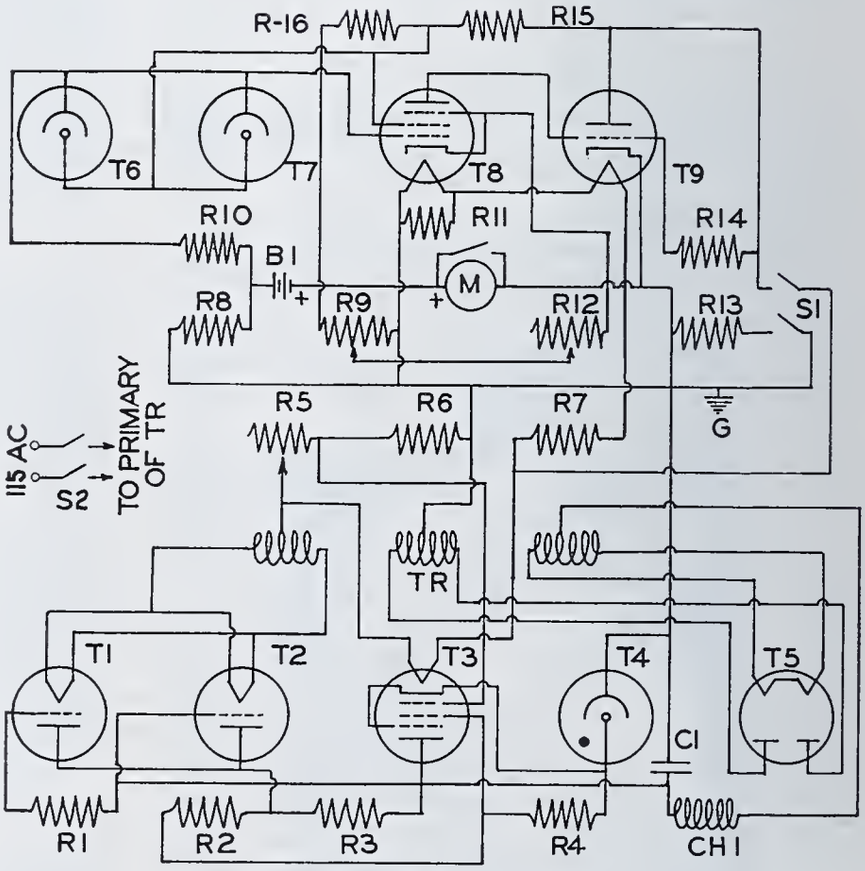

FIGURE 2. DIAGRAM OF CONNECTIONS

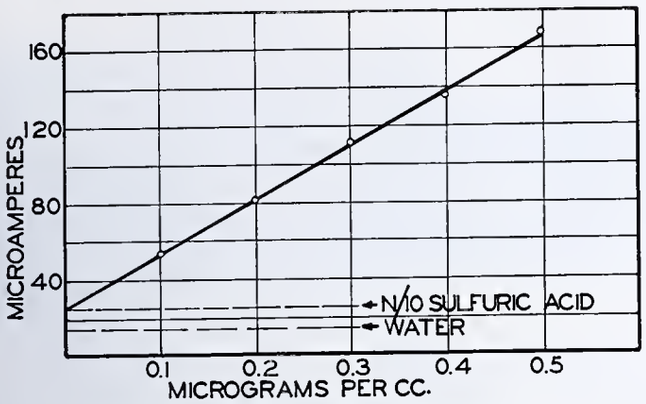

FIGURE 3. GRAPH OBTAINED WITH FLUORIMETER USING QUININE SULFATE IN 0.1 *N* SULFURIC ACID

T74C29 choke; *T*1, 2A3; *T*2, 2A3; *T*3, 12SJ7; *T*4, VR-105-30; *T*5, 5Z3; *T*6, R. C. A. 929; *T*7, R. C. A. 929; *T*8, 12J7-GT; *T*9, 12SF5. See Figure 2 for diagram of connections.

The ultraviolet lamp is a Westinghouse BH-4, 100-watt with a natural red-purple bulb. A special transformer and socket for the lamp are also needed. The filters are Corning 5 cm. (2 inches) square, polished; those used for the curve shown in Figure 3 were No. 4308, 3.14 mm. thick, and No. 3389, 1.51 mm. thick. The cell used for Figure 3 had a capacity of 25 ml. and was made of glass with one window of ultraviolet transmitting glass to permit a larger amount of the ultraviolet light to enter the liquid. Many liquids will fluoresce enough to give satisfactory readings when ordinary glass cells are used.

Acknowledgment

The writers wish to acknowledge the assistance of members of the Department of Biologlcal Chemistry of the University of Cincinnati, who carefully prepared all the solutions used in testing the fluorimeter.

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A Constant Pressure and Flow Ratio Regulator for Continuously Mixing Two Gases

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A FLOW regulator was required which would introduce two components of a gas mixture into a reaction chamber at a constant predetermined ratio of flows and a constant reaction pressure, regardless of variations in reaction chamber pumping speed, rate of chemical reaction, initial pressures, and differential consumption rates of reactants.

The following system meets these requirements, and while it was designed for flows of each gas in the range 0.40 to 20.0 cc. per second at 50 to 200 mm. of mercury, it should be equally applicable to other flow and pressure ranges. The design is such that sparking regulator contacts are not exposed to the mixed gases. Thus even with a pair of gases which form an inflammable mixture, there is no danger of spark ignition.

A constant flow ratio is maintained on the principle that the ratio of flows through two resistances will remain constant regardless of changes in absolute values of pressure or flow, providing the ratio of the mean pressures remains constant. For a reactor feed system, where the reactor pressure is the common final pressure of the two gas inlet systems, this condition can be readily maintained by establishing and maintaining an equality of pressures between the inlet lines at some other point, for the ratio of mean pressures in the lines between this point and the reactor thus remains constant at 1. This can be accomplished by a control U-tube manometer.

Description

A schematic diagram of the flow system and the electrical circuits is shown in Figure 1. The control manometer, *F*, has two fixed electrical contacts on one limb. Valves *A* and *B* between the limbs of manometer *F* and the flowmeters, *M* and *Q*, provide the desired adjustment of ratio of resistances in the lines between *F* and the reactor. *E* is an open manometer, for control of pressure; it has a movable double contact arm in the open side. The contacts are slightly displaced vertically in *E* and *F*. Both manometers are sufficiently long to allow evacuation of any portion of the system, and both have a third contact sealed into the bottom.

All manometer tubes should be at least 10 mm. in inside diameter to prevent excessive sticking of the mercury meniscus. Manometer *F* is filled with mercury to a position midway between the contact points when both sides are balanced.

The motor-driven valves *C* and *D*, details of which are shown in Figure 2, are a modified form of the valve described by Fowler (*1*), and consist of a thin-walled brass tube, flattened and bent into a U. The valve is opened or closed by expanding or contracting the U by means of a rotating lead screw.

For other flow ranges, different sizes of tubing from that shown will be necessary. An ordinary needle valve is satisfactory for the higher flow speeds.

The lead screw of the U-type valve, or the stem of the needle valve, is turned by a 4 r. p. m. Telechron motor, Type C2M. By reversing one of the pole pieces in each of the Telechron motors, the motors can be made to turn in either direction.

The choice of valve operating speeds will be governed mainly by the resistances of the connecting gas lines and by the volume of the reactor. These lines should be as short as possible for the most rapid compensation without hunting. In the present system, it was necessary to place the reactor about 360 cm. (12 feet) from the control apparatus and to make the lines of 0.6-cm. (0.25-inch) inside diameter tubing; thus, a rather slow valve operating speed was required. The reactor had a volume of about 500 ml. These figures may serve as a starting point for the selection of speeds for other conditions.

Parts 1, 2, 3, and 4, in Figure 1, are battery-operated relays which require not more than 20 milliamperes current to energize, and have load capacities of 0.2 ampere. Double-pole double-throw relays are used for 1, 2, and 4, and a single-pole double-throw relay for 3. Part 6 is a double-pole double-throw switch with a neutral, or "off", position, for changing from manual to automatic control of the valves. Group 7 consists of four double-pole single-throw push-button switches for manual control of both motors in either direction.

Testing the Circuit

The circuit may be tested by shorting manometer contacts, as indicated in Table I, and observing the response of the valves.

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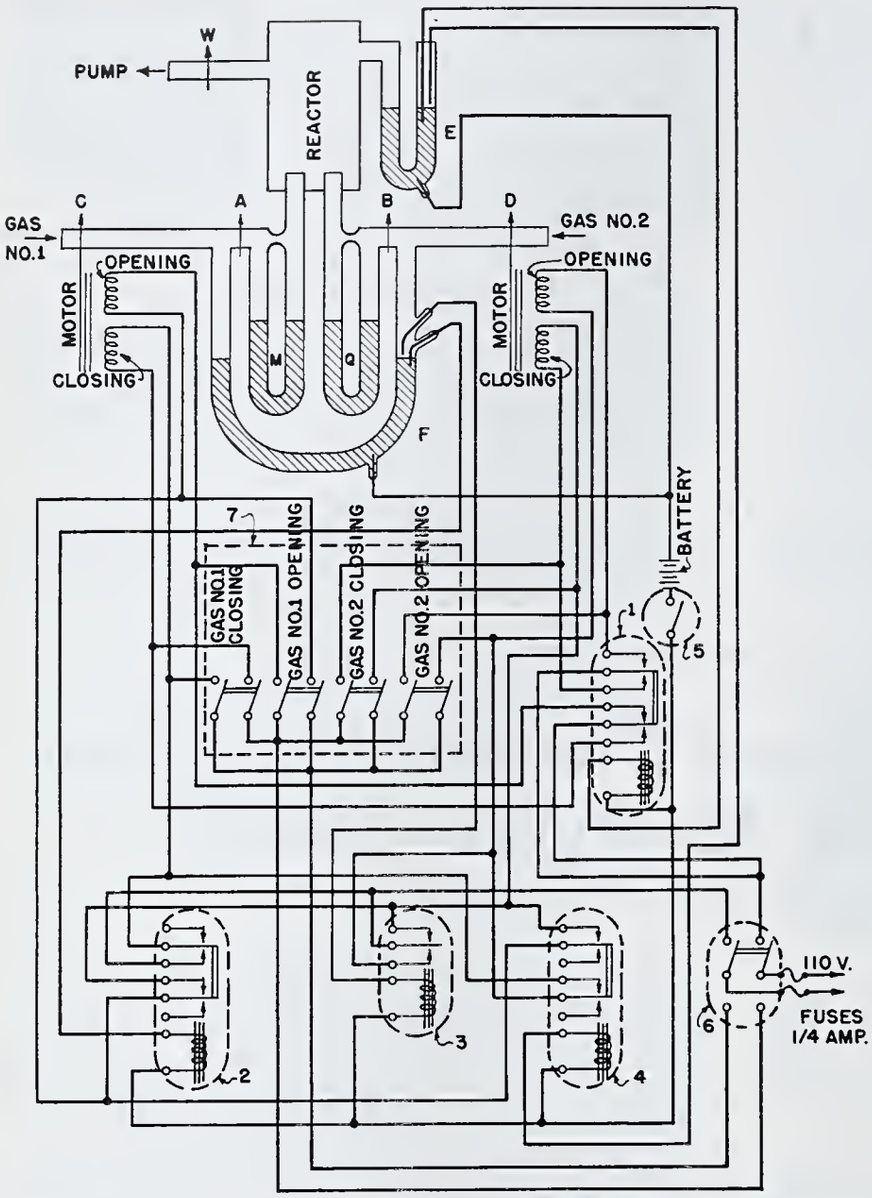

FIGURE 1. DIAGRAM OF CONSTANT PRESSURE AND FLOW RATIO REGULATOR

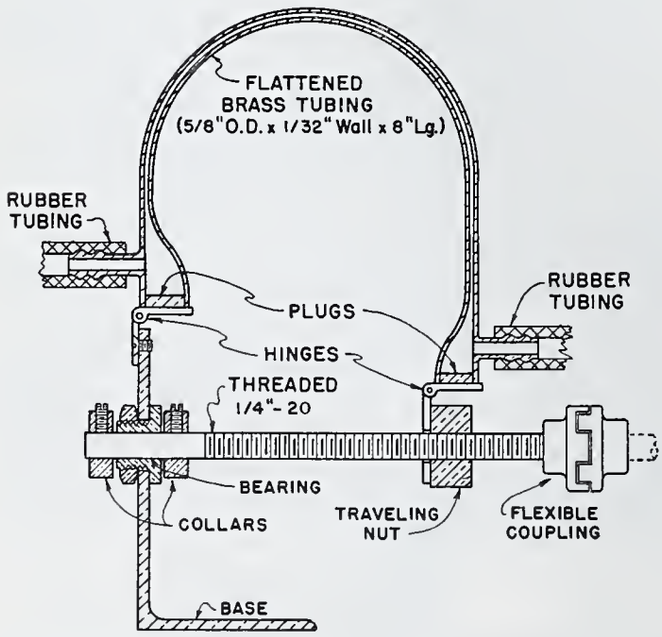

FIGURE 2. MOTOR-OPERATED VALVE FOR SMALL FLOW RATES

Operation

Valves *A* and *B* are first opened completely, battery switch 5 is closed, and switch 6 is turned to "manual" position. With any arbitrary pumping speed and pressure, the flow ratio is adjusted by means of the push-button controls, 7. This will probably unbalance manometer *F*. The flow rate of the reactant which has the lower pressure is noted, and then valve *A* or *B*, whichever is on the low pressure side, is gradually closed, and the motor-driven valve on the same side is opened. This process is continued until, by a series of approximations, manometer *F* is balanced and the original flow rate is restored. The desired reactor pressure is adjusted by means of the movable contacts in *E*. Switch 5 can now be changed from "manual" to "automatic", and the system will begin regulating. If the total flow speed requires correction, this can be accomplished by adjustment of valve *W*. This will not interfere with the established values of flow ratio or pressure. In subsequent runs, the original conditions will be automatically restored, providing valves *A*, *B*, and *W* are not changed.

Application

The unit has been used to control the input of two reactants into a chamber in which rapid reaction can be initiated; such reaction results in a sudden pressure change of some 30 per cent. The regulator satisfactorily restores the pressure to its original value by changing the flow speed of the component gases, without changing the component ratio. Even with the long lines which the author used to connect reactor and regulator, the time to compensate for such large pressure changes was only on the order of a minute, after which compensation for minor fluctuations was much more rapid and was generally made to a constancy, in both pressure and flow speed, of about 1 per cent. At the lower end of the range of flow speeds hunting set in to some extent, but this could be readily eliminated, if desired, by reducing the speed of the valve motors, by using interchangeable lead screws of lower pitch, or by reducing the length of the flow lines between regulator and reactor.

TABLE I. MANOMETER CONTACTS

Conditions		Contacts[b]					
		Manometer *F*		Manometer *E*		Motor Valves	
Flow ratio[a]	Pressure	Upper	Lower	Upper	Lower	Gas 1	Gas 2
Gas 1 high	High	In	In	In	In	Closes	..
Gas 2 high	High	Out	Out	In	In	..	Closes
Balanced	High	Out	In	In	In	Closes	Closes
Gas 1 high	Balanced	In	In	Out	In	..	Opens
Gas 2 high	Balanced	Out	Out	Out	In	Opens	..
Balanced	Balanced	Out	In	Out	In	..	..
Gas 1 low	Low	Out	Out	Out	Out	Opens	..
Gas 2 low	Low	In	In	Out	Out	..	Opens
Balanced	Low	Out	In	Out	Out	Opens	Opens

[a] "High" and "low" refer to required flow.
[b] "In" and "out" indicate that particular contacts are in or out of mercury, and therefore corresponding circuits should be closed, or opened, respectively.

Acknowledgment

The author wishes to thank George C. Eltenton for valuable suggestions.

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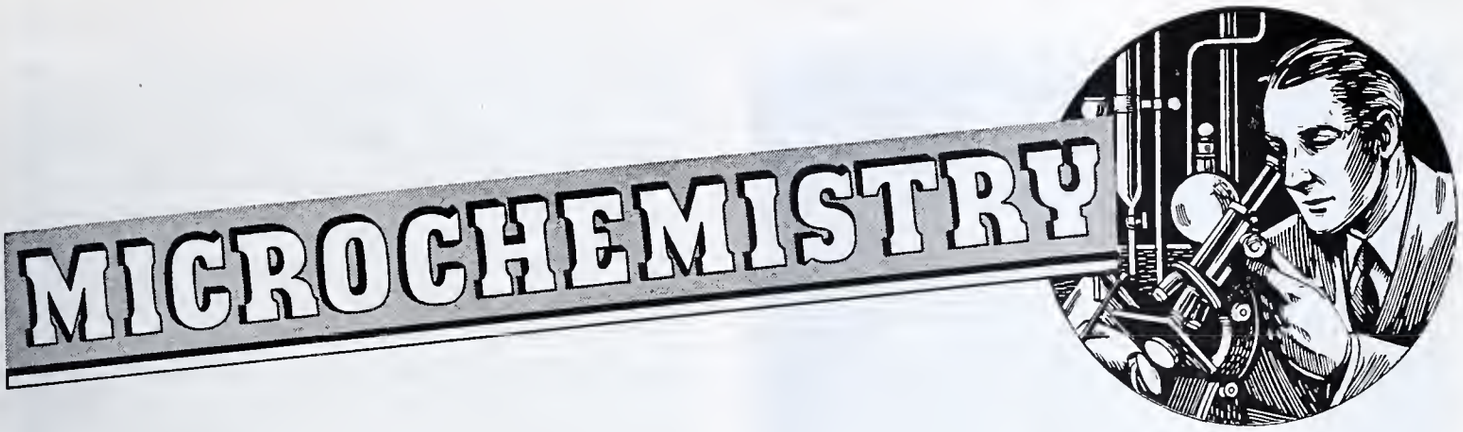

Analytical Patterns in the Study of Mineral and Biological Materials

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DURING the past half century numerous investigators have devised printing methods whose primary object was to ascertain the distribution of a particular component in the samples studied by them. These isolated individual methods include autoradiography employed by mineralogists in the study of radioactive inclusions in rocks (*36*, *50*), the methods of sulfide (*6*), phosphide (*37*), and iron oxide (*57*) printing employed in metallurgical laboratories, and the more generally applicable electrographic method devised by Glazunov for the study of liquations in alloys (*14–16*, *18–26*).

When an effort is made to correlate these diverse techniques, one can visualize the development of a system of two-dimensional analytical chemistry in which a selected plane of the massive sample plays a role similar to that of the representative sample of powdered material in classical analysis. The primary object of such a system of analysis would be to reveal the mode of distribution of the chemical components as an adjunct to the established methods of qualitative and quantitative analysis. This analytical approach frequently facilitates the detection of minor constituents where these have crystallized as distinct units and permits a fairly complete qualitative analysis of the major components without causing appreciable injury to the prepared surface. Each component is characterized by an "analytical pattern" which may be described as an imprint of the surface on an external medium that can be interpreted in terms of its chemical composition as a result of the controlled mechanism of its formation.

TABLE I. CLASSIFICATION OF ANALYTICAL PATTERNS

A. Produced by physical agencies
   1. Autoradiograph
   2. Autoluminograph
   3. Magnetograph
B. Produced by chemical reactions
   1. Contact print
   2. Electrographic print

Classification of Patterns

The analytical patterns can be classified in two distinct groups as represented in Table I. In the first classification are those patterns made possible by some intrinsic physical property of one of the components, such as radioactivity, characteristic luminescent effects, or the presence of marked paramagnetism. These patterns are characterized by the absence of any loss of sample whatsoever during their execution and all enjoy the simplicity of the readily available photographic emulsion as their recording medium. Such simple physical mechanisms are available for only relatively few of the elementary constituents. In general it is necessary to strip a film from the surface by some appropriate solvent or electrolyte and then to make its components evident through the use of selective chemical reactions.

When the stripping is effected by means of solvents the processed pattern is commonly known as a contact print. In the case of electrically conducting materials the surface film can be transposed electrolytically and the finished print is termed an electrograph (*11*, *18*). These chemical methods of stripping do not involve the removal of a complete plane, such as would be secured by cutting the specimen with a microtome. Such uniform sampling could be effected only in the case of perfectly homogeneous metals. In general, when a solvent acts on a complex surface a preferential solubility usually exists for one or more of the components. This factor is of considerable importance, as by the judicious selection of a stripping agent it is often possible to improve the specificity of a given analytical pattern.

Of the patterns produced by physical agencies, the autoradiograph is by far the most important. The alpha-rays emitted by radioactive elements activate a photographic emulsion the same way as ordinary light rays. This actinic action was applied by Mügge (*55*) as a means of studying the distribution of thorium and uranium compounds in minerals by contacting the specimen with an emulsion for a suitable period of time and noting the blackening produced on subsequent development. A thin sheet of black paper or aluminum foil is usually placed between the plate and the specimen to avoid direct chemical action or to prevent luminescent light from activating the emulsion and thereby interfering with the specificity of the autoradiographic pattern.

The period of exposure varies with the activity of the specimen and the speed of the film. The writer has found the small 3 × 4 cm. x-ray film, in common use by dentists, convenient for test purposes. Although it is possible to secure an image by simply placing the specimen on the film ensemble, better definition results if the specimen is exposed in a light-tight can directly against the film removed from its protective jacket of paper and

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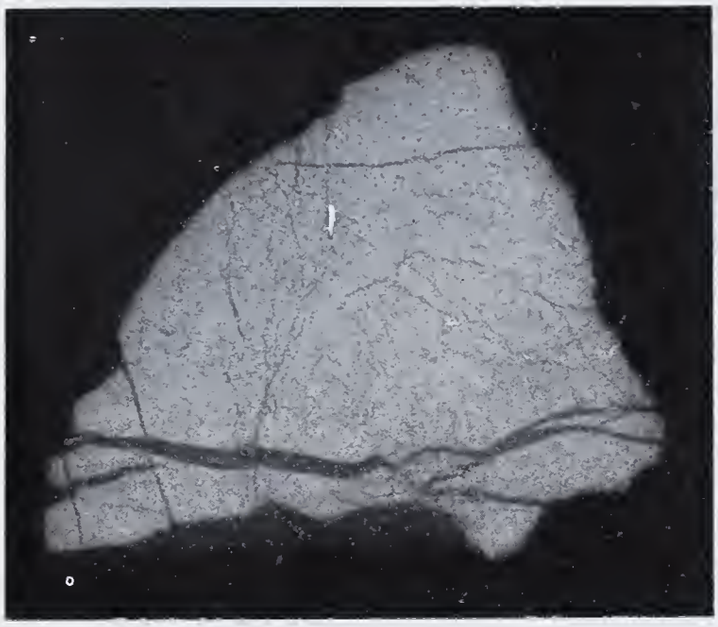

FIGURE 1. AUTORADIOGRAPH OF PITCHBLENDE FROM JOHANNGEORGENSTADT, BOHEMIA

Area enlarged 50-fold, exposure 24 hours. Pattern reveals presence of very fine veins of nonradioactive material.

lead foil. After overnight exposure the film is developed with a solution consisting of one part of Eastman print developer D-72 and two parts of water. The film records considerable fine detail of the radioactive surface, which is best observed on an enlargement print (Figure 1).

This mechanism was employed by Lacassagne (*51*) as a means of studying the distribution of polonium salts injected into the blood stream of animals. After autopsy the organs were fixed and thin sections were contacted with photographic plates. The intensity of the developed images served as a measure of the distribution of the polonium and the prints frequently exhibited the cellular detail characteristic of the tissue. The localization of lead in animal organs has likewise been established by Lomholt (*53*) and independently by Behrens and Bauman (*7*), using radio-active isotopes of lead as tracers. The autoradiographic techniques has also proved fruitful in the study of occlusion within large crystals grown in solutions containing traces of radioactive ions (*33*), and in investigations of the behavior of radioactive elements in alloys during melting, rolling, and wire-drawing processes (*63*, *64*).

The field of autoradiography has been extended with the discovery of methods for synthesizing radioactive isotopes of the lighter elements. These radioactive isotopes serve as indicators for tracing the diffusion of the normal elements found in living structures and the technique can be employed wherever a radioactive isotope of suitable life can be synthesized for the element in question.

The assimilation of phosphorus in plants and the distribution of iodine in thyroid tissue have thereby been recorded by Hamilton (*34*). The same mechanism has been applied in the identification of phosphorus liquations in steels, during the manufacture of which some radio-phosphorus has been added (*66*). The method has also been applied to the study of minerals that are not naturally radioactive. By exposing polished sections of manganese ores to electron bombardment in a cyclotron, Goodman and Picton (*28*) have secured autoradiographic patterns of the normally nonradioactive specimens owing to the formation of radio-manganese. Goodman (*27*) has subsequently demonstrated that this novel autoradiographic technique can also be applied in the study of the ores of tungsten, gold, arsenic, sodium, potassium, and phosphorus.

Among the other physical properties of matter which can be utilized on occasion in the formation of patterns having analytical significance are those of luminescence and magnetism. The latter property is sufficiently pronounced only in the case of metallic iron and some of its mineral occurrences, such as magnetite and pyrrhotite, and can be demonstrated by sprinkling iron filings on a photographic printing paper placed over the magnetized specimen. This property is of little practical importance, as an iron pattern is more readily made by chemical methods. The writer has found luminescent properties serviceable in studying the homogeneous character of mineral specimens. After exposure to ultraviolet light, many substances continue to luminesce for a considerable period after their removal from the source of initial excitation. Although this afterglow is usually feeble and can be observed only in perfect darkness, it is of sufficient intensity to activate a photographic plate when it is brought in direct contact with it. A relatively brief exposure of 2 or 3 minutes suffices, so that there is very little danger of confusing the resultant picture with the pattern produced by any radioactive constituents that might be present. This simple mechanism furnishes a permanent record of the evanescent

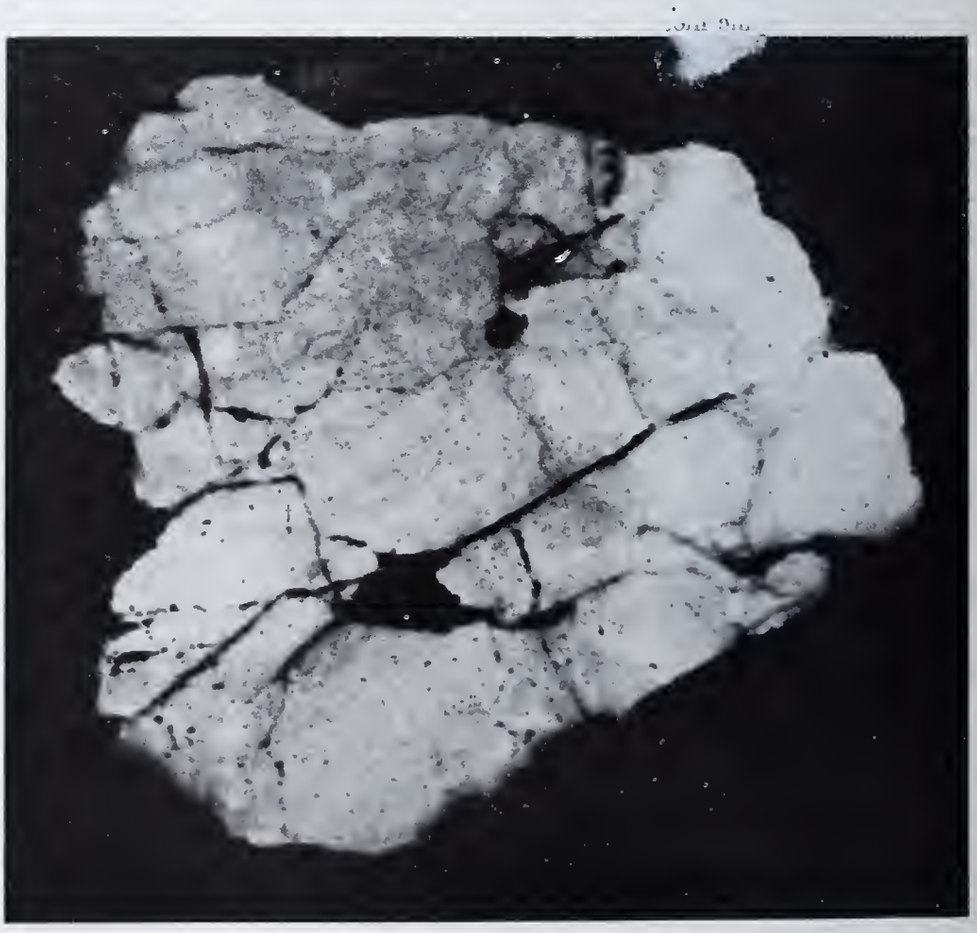

FIGURE 2. AUTOLUMINOGRAPH OF ANTOZONITE, A VARIETY OF FLUORITE FROM ONTARIO, CANADA

Area enlarged 16-fold, exposure 2 minutes. White luminescent areas are produced by calcium fluoride; black nonluminous veins reveal presence of intrusions, which on analysis proved to be limestone and silica.

luminescent effect and is particularly useful in the study of occluded foreign bodies in minerals which exhibit the property (Figure 2).

An electrical method of reproducing surfaces with low relief, such as leaves of plants and coins, has been described by Prat and Schlemmer (*59*).

In this method, termed electrography, the specimen is placed in direct contact with a dry photographic emulsion and the unit is sandwiched between two iron plates connected to the secondary of a high-voltage spark coil. After a brief exposure to the electric discharge and development of the plate, an image is produced outlining the ridges of the specimen and its surrounding corona discharge.

This process does not yield an analytical pattern, since the print furnishes no information about the chemical composition of the specimen. It is of value, however, as a means of recording the path of a high-voltage pulsating current through the material under investigation, and suggests an extension of the electrographic method to samples composed of conducting particles embedded in a nonconducting matrix.

The sulfur print method for exhibiting the distribution of sulfides in iron alloys is one of the earliest examples of an analytical pattern produced by chemical reaction (*6*).

This imprint is secured by pressing the polished section against a sheet of photographic paper that has been moistened with 2 per cent sulfuric acid and maintaining the contact for about 2 minutes. The acid causes the evolution of hydrogen sulfide, which reacts with the silver halides to form dark brown silver sulfide. A permanent print is secured by rinsing off the acid, fixing in a hypo bath, and washing in running water. The contact printing method has since been applied to the identification of phosphate minerals in rocks by Leitmeier (*52*), to copper, nickel, cobalt, and iron oxide liquations by Niessner (*58*), and in the investigation of mineral sections by Gutzeit (*31*).

The electrographic method of printing, devised by Glazunov (*17*) and independently by Fritz (*11*), is essentially a reversal of the principle employed in electroplating.

In this method an absorbent paper moistened with a suitable electrolyte is inserted between a cathode of inert material such as aluminum and the polished face of the alloy which serves as the anode. This unit is connected to a battery of dry cells and a current is allowed to flow for several seconds. During this electrical exposure ions leave the surface of the specimen and migrate into the permeable medium, where their presence can be made manifest by reaction with selective reagents. The simplicity and elegance of this technique soon attracted many devotees, who applied the method to the solution of problems in metallurgy (*1, 35, 45, 46*), mineralogy (*31, 47*), and biology (*67*).

The electrographic method is of course applicable only to materials that are conductors of the electric current. While the number of conducting minerals constitutes only a minor fraction of all the known species, many of the conducting minerals are fairly common and are often ores of economic importance, such as galena, pyrites, and sulfide minerals in general. In the biological field the method is applicable to the localization of those constituents which are normally present within the tissue in an ionic state.

The Media

The ideal medium for the execution of patterns by chemical agencies is one that is structureless, is preferably transparent, conducts the electric current, is resistant to the action of the etching and developing reagents, and finally does not become distorted while drying. While no one of the media listed in Table II meets all the requirements, a good approximation is usually available to meet the demands of individual patterns.

Absorbent smooth-textured papers are satisfactory as printing media only when an image of the macrostructure of the specimen suffices for the study. Any attempt at magnifying the pattern brings out the fibrous structure of the medium and prevents observation of detail. The outstanding advantage of this medium is its extreme resistance to the action of concentrated volatile acids, and if a prehardened paper is selected such as C. S. & S. 507, it will exhibit little shrinkage after washing.

Table II. Media Suitable for Analytical Patterns

1. Hardened absorbent papers
2. Gelatin-coated paper
3. Gelatin plates and films
4. Thin plaster castings
5. Transparent water-permeable plastics

Dense paper coated with a film of pure hardened gelatin makes a satisfactory medium for contact and electrographic work which permits the resolution of fine detail on the developed pattern. Such paper, free from silver halides, can be obtained from manufacturers of photographic paper. The dense backing of the gelatin-coated paper is rendered electrically conducting by allowing the sheets to soak in a bath of a suitable electrolyte for at least 10 minutes. The gelatin coating tends to hydrolyze on prolonged action of strong mineral acids; nevertheless the medium can be employed for contact printing, provided the acids are cold, and are diluted with equal volumes of water and the paper is washed shortly after the development process. The opaque backing necessitates the observation of the print by reflected light and requires a microscope provided with a vertical illuminator if photomicrographs of the pattern are to be made.

Transparent nonconducting gelatin-coated films suitable for contact printing can be made by extracting the silver halides from photographic film and hardening the gelatin coating. This is accomplished by placing the film in a chrome alum hypo bath for 20 minutes, washing in running tap water for about 1 hour, agitating in distilled water for about 15 minutes, and finally drying in a dust-free atmosphere. The chief advantage of this medium over gelatin paper in contact printing resides in its transparency. This permits the projection of the print onto a screen and its microscopic examination by means of transmitted light.

In an effort to develop a transparent medium that also conducts the electric current, it was discovered that certain varieties of commercial cellophane, that have not been waterproofed, serve

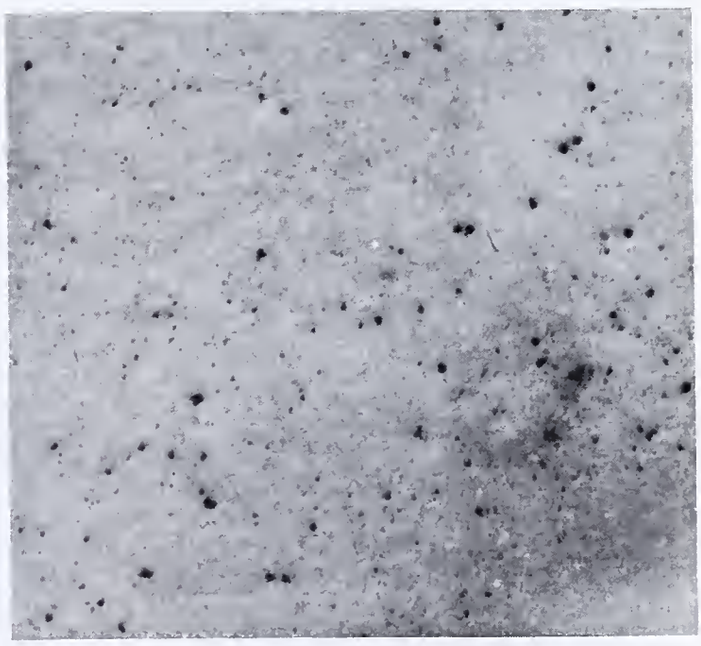

Figure 3. Microphotographic Detail from Lead Pattern of Section of Bronze, ×20

Bronze contacted for 15 seconds against plaster casting moistened with 1 per cent acetic acid. Lead entering solution is immediately fixed on medium as lead sulfate, which is rendered visible by conversion to black lead sulfide on exposure to hydrogen sulfide vapors. Microscopic examination of pattern demonstrates that lead is segregated in minute spherules within bronze matrix.

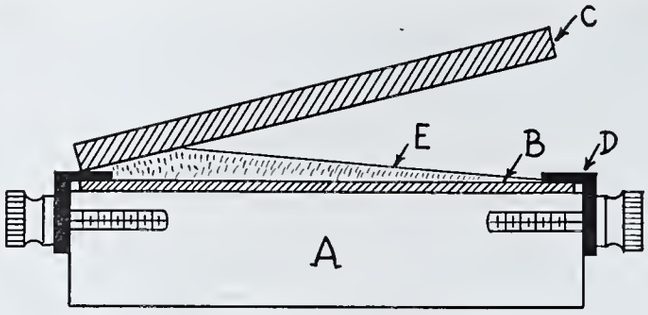

FIGURE 4. CROSS-SECTIONAL VIEW OF MOLD FOR CASTING THIN SHEETS OF PLASTER OF PARIS

A. Hardwood block
B. Glass backing plate
C. Glass surfacing plate
D. Angle railing machined from metal or plastic
E. Slurry of plaster of Paris

as conductors of the second class when moistened with an electrolyte. The thin film is subject to shrinkage while drying so that it is serviceable only in the examination of macrostructures. The distortion of the image can be minimized by preshrinking the cellophane in a water bath and drying prior to its being moistened with an electrolyte. This medium is particularly adapted to the study of the iron pattern in conjunction with potassium ferrocyanide as the developing agent, as the blue image exhibits no bleeding during the washing process, in marked contrast to the behavior on the surface of most other media.

The thin castings of plaster of Paris developed by the writer for producing sodium and potassium patterns of vital tissue (*67*) have unique properties which merit attention. When the plaster casting is moistened with water it furnishes its own electrolyte in the form of a dilute solution of calcium sulfate. By virtue of the fixed source of sulfate ion, the medium serves as a reagent for the fixation of lead sulfate. These inherent properties offer a very simple approach to the study of lead patterns (Figure 3). Water-insoluble reagents such as zinc sulfide, barium carbonate, aluminum oxide, etc., are readily incorporated into the plaster sheet during the casting process. This makes the medium of value not only in electrographic work but also in many other analytical techniques where a fine-grained capillary matrix sensitized with such reagents is of importance, as in spot tests, in capillary analysis, and in the execution of microchromatographic separations of complex organic materials.

The following process is employed by the writer for the preparation of thin plaster sheets that are substantially free from minute air bubbles:

The glass backing plate (Figure 4, *B*) is cleaned with alcohol, placed in the mold, and rubbed dry with a soft lintless paper. To fill a cast having a total volume of 20 ml., 30 grams of plaster are added to a 50-ml. beaker containing 20 ml. of distilled water and the mixture is rubbed into a uniform paste with the aid of a rubber-tipped rod. The thin paste is immediately transferred to the center of the backing plate and the entire mold is bounced against a sponge rubber mat with sufficient force to cause the mix to spread over the greater area of the plate. The surface is then aerated momentarily with ethyl alcohol vapor, and the mold is again briefly agitated. The vibration brings the air bubbles to the surface, where they can be destroyed by the lowering of the surface tension caused by the alcohol vapor. This process is continued until the ascension of the occluded air ceases, but should not be prolonged for more than 2 minutes.

The unit is now removed from the rubber mat and the mold is tilted sufficiently for the mix to flow towards the lower rail. The heavy surfacing plate, *C*, is then placed on the lower rail and rotated downwards with a slow firm motion until it is brought in contact with the opposite support. The excess plaster is wiped off, and about 15 minutes later, when the plaster has set, the upper plate is removed by wedging a thin blade under its corners. The rails are removed and the bottom plate with its adhering sheet of plaster is set aside to dry at a temperature not exceeding 80° C. It is good practice to make several castings late in the afternoon and to permit the sheets to dry slowly at room temperature overnight. When thoroughly dry, the casting separates readily from its backing plate. The sheets are then cut into squares of suitable size by making scratch lines with a razor blade and bending the plaster along the lines of demarcation.

The thickness of the plaster castings can be varied from about 0.4 mm. and up by selecting rails of suitable gage and grinding them down to the desired height. It is well to standardize on sheets 1 mm. thick, as these possess adequate mechanical strength and are sufficiently translucent to permit the observation of colored compounds by transmitted light. In the preparation of specially sensitized castings with water-insoluble reagents, the water employed in the casting process is replaced by an equal volume of a thin suspension of freshly precipitated and washed reagent. In making plaster sheets for chromatographic analysis, about 20 per cent of powdered alumina or dry precipitated chalk is added to the plaster powder and mixed with the water as in the standard casting procedure. The plaster medium itself frequently serves as a chromatographic adsorbent.

Preparation of the Specimen

Minerals sectioned by any one of the standard procedures employed in petrographic laboratories for microscopic examination of their structure are also suited for the preparation of their analytical patterns. The equipment necessary for mounting specimens in plastics may not always be available and the following simple and inexpensive procedure will be found adequate.

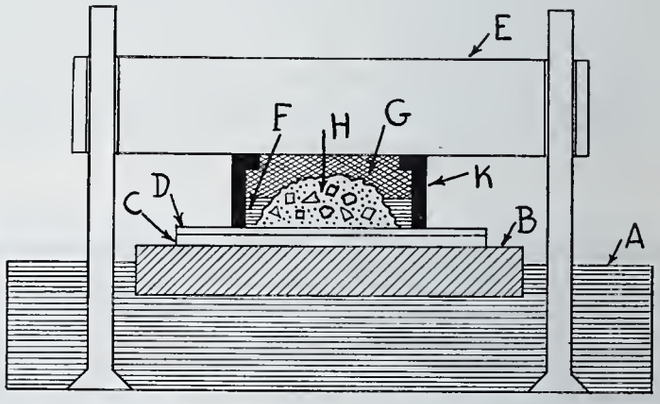

FIGURE 5. PRESS FOR EXECUTION OF ANALYTICAL PATTERNS

A. Wooden block with groove lined with lead foil
B. Electrode sliding on *A*
C. Paper pad similar to C. S. & S. No. 470
D. Medium for recording pattern
E. Lead casting
F. Sealing wax
G. Fusible metal
H. Embedded specimen
K. Plastic screw cap

The sample is first trimmed to fit into a plastic screw cap about 3 to 4 cm. in diameter, the greater part of whose top has been drilled away, so that substantially only a threaded wall remains (Figure 5, *K*). An approximately plane surface is then prepared by grinding the specimen on a glass plate covered with a slurry of 100-mesh Carborundum powder and water until the desired surface is exposed. The mineral is washed free from grit, dried, and placed in an oven at 110° C. on a metal plate covered with a sheet of sized paper. After about 5 minutes the plate is removed from the oven, the warm specimen is covered with the screw cap, and molten sealing wax is poured through the opening until the cap is completely filled. Commercial sealing wax may be used, but it is best to prepare one free from inorganic fillers by heating a mixture of 60 grams of flaked shellac, 45 grams of rosin, and 23 ml. of turpentine until vigorous bubbling sets in (*61*).

When the unit cools to room temperature the mounting is separated from the paper and the grinding is continued until the surface of the mineral is exposed. The section is then polished on fine emery paper until the scratches from the grinding process are removed. In mounting minerals that conduct the electric current, only the lower half of the specimen is embedded in wax. After solidification, the upper half is scraped free from any ad-

hering wax and tarnish and the screw cap is filled with a low-melting alloy. One suitable for this purpose can be prepared by melting together 50 parts of bismuth, 25 parts of lead, and 12.5 parts each of tin and cadmium.

Biological materials require no special mounting prior to their electrographic analysis when the constituents exist as ions in the tissue. A parallel-faced slice about 4 mm. thick is cut from a fresh organ and is drained on lintless filter paper and then exposed to the sensitized emulsion. The orientation of non-ionic inorganic constituents can be established by destroying the organic matter in a thin section and making contact prints of the residual ash. The slow combustion of a thin section of tissue mounted on a glass slide results in an ash skeleton in which the inorganic constituents are deposited exactly as in the original tissue. This pattern, analogous to the total ash determination in classical analysis, is known as a spodogram (*60*). The ash resulting from the microincineration of the tissue is in many respects akin to a polished mineral section and its constituents can be studied by the methods of contact printing.

Preparation of the Pattern

The apparatus employed in the execution of the patterns (Figure 5) is essentially a press for maintaining uniform contact of the specimen with the printing medium.

A metallic foil, *A*, serves to make electrical contact with a sliding slab, *B*. This electrode may be of any inert metal such as aluminum, platinum-clad steel, or heavily silver-plated brass. Hiller (*38*, *41*) and Gutzeit (*29*) employ as electrode material a layer of sponge rubber or felt covered with an envelope of aluminum foil. The alkali hydroxides formed during the electrolysis tend to corrode the cathode, particularly if it is aluminum. Any visible corrosion should be removed by burnishing the surface with fine steel wool; otherwise it will constitute an added irregular resistance and prevent the formation of a perfect image. The paper pad, *C*, between the cathode and the medium, *D*, equalizes the pressure on the face of the specimen and facilitates uniform contact. The pad is rendered conducting with an appropriate electrolyte and is drained of excess fluid before being placed on the electrode. The medium is likewise moistened with electrolyte, and buffering and developing agents, and thoroughly blotted between absorbent paper. The specimen is then brought in contact with the medium, by lowering it at an angle, so as to avoid the inclusion of air bubbles. The electrode with its assembly is transferred to the groove in the base of the press, so that the specimen is centered with reference to the lead casting, *E*, which when lowered subjects the specimen to a reproducible pressure. This casting should weigh about 500 grams, and when necessary, the pressure can be augmented by placing added weights. In making autoradiographic prints a small C-clamp suffices for contact of the specimen against the film.

When the surface is to be stripped electrolytically the electrodes are connected to the appropriate terminals of a 22.5-volt B battery in series with a variable rheostat of about 500 ohms' resistance and a meter having a range of about 100 milliamperes. Although the electrode potentials of most elements is below 3 volts, it is necessary to apply a much higher voltage in order to overcome the resistance of poorly conducting minerals and the internal resistance of the medium. The duration of the current seldom exceeds 30 seconds, the optimum exposure being determined by trial on sucessive prints. The intensity of the current, and its duration can be approximated in the case of fairly homogeneous specimens with the aid of Faraday's second law

$$ti = 96{,}500\, Adn/W$$

In this expression i is the current in amperes, t is its duration in seconds, A is the area of the polished surface in sq. cm., W and n are the atomic weight and valence of the particular ion whose pattern is to be rendered, and d is the weight of the element in grams per sq. cm. that must be electrolyzed in order to produce the desired intensity of color on development.

Experience with quantitative drop reactions on paper shows that in general 50 micrograms of most of the metals produce brilliantly colored products when the reaction is confined to an area of 1 sq. cm. (*70*). Using this density factor the equation reveals that when copper is electrolyzed an exposure of 15 milliamperes for 10 seconds per sq. cm. of surface will result in a print of good color intensity. This is in fair agreement with actual working exposures resulting in satisfactory patterns of copper alloys and ores. Since this equation does not take into consideration the electrical energy expended in decomposing the electrolyte, the calculated exposures must be considered as first approximations useful in gaging the initial trial exposure.

In exposing a specimen composed of several different mineral species, the current passing through the individual crystals will vary with their resistivity and cross-sectional area. Under these circumstances the sample comprises a series of resistances connected in multiple, and the current may be carried almost entirely by the component of highest conductivity, with the result that the pattern will be underexposed with respect to the poorly conducting constituents. Stripping of a more uniform character is achieved by moistening the medium with a mineral acid instead of a neutral electrolyte (*41*).

After exposure, the image is rendered visible by treating the medium with a reagent solution that produces a characteristic insoluble colored compound with the ion in question. Since the number of specific reactions are rather limited, interfering ions brought into solution during the stripping process must be removed prior to development. To prevent diffusion of the element whose pattern is being processed, it is good practice to fix the ion in the medium as a sparingly soluble compound. This principle is illustrated in the method for the formation of chloride patterns (*67*).

The medium consists of gelatin-coated paper impregnated with silver chromate. On electrical exposure the chloride ion is fixed as insoluble silver chloride, and the print can be washed with dilute nitric acid in order to leach out excess reagent and other compounds that enter the emulsion during electrophoresis of the tissue. After washing in water, the colorless image is rendered visible by reducing the silver chloride to metallic silver with the aid of a photographic developing solution. A typical example of the resultant chloride pattern is shown in Figure 6.

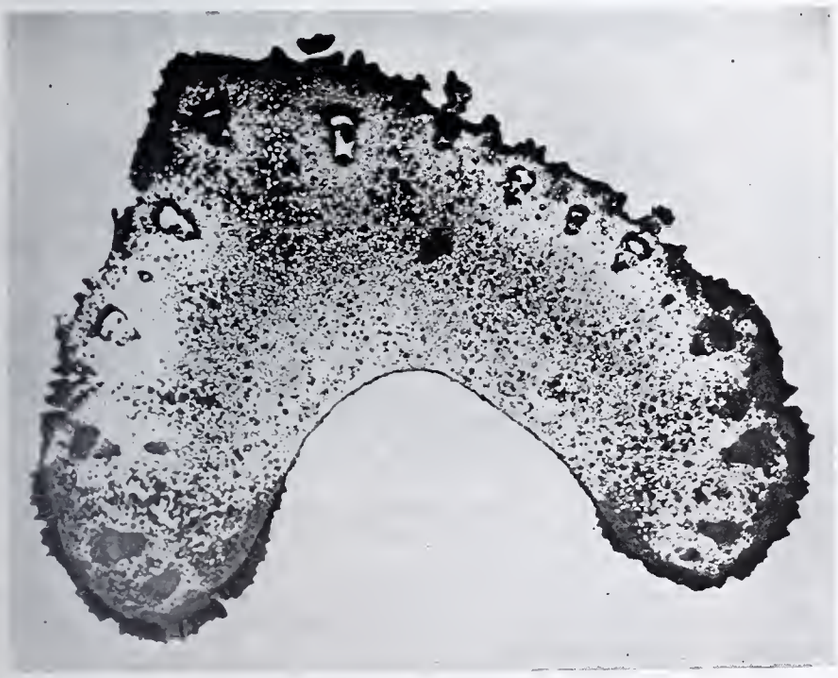

FIGURE 6. CHLORIDE PATTERN OF CROSS SECTION OF CELERY

Area enlarged 20-fold, exposure 5 ma. for 15 seconds. Pattern exhibits physical structure of cell walls and demonstrates concentration of chloride ion in epidermal wall, vascular bundles, and central collenchyma tissue.

In contact printing, solution of the surface film is effected by the action of dilute mineral or organic acids, ammonia, and alkalies. Refractory ores can often be attacked by moistening the medium with a mixture consisting of 1 part of nitric acid, 1 part of hydrochloric acid, and 2 parts of water. In specific cases a selective stripping for one or more components can be achieved through the action of potassium cyanide (silver), ammonia and hydrogen peroxide (arsenic), tartaric and phosphoric acids (antimony), bromine water (gold), sulfurous acid (manganese), etc. The optimum method of attack for any particular pattern depends on the nature of the mineral and is best established by preliminary tests.

As an example of the applications of the contact printing technique, consider a mineral section containing copper, nickel, cobalt, iron, and sulfur as principal components. A joint pattern of the copper, nickel, and cobalt can be rendered by bringing the polished surface into contact with gelatin paper moistened with equal volumes of ammonia and water. These elements form soluble ammines and their presence is made manifest by developing the medium with a saturated alcoholic solution of dithiooxamide (*10*) which forms a dark green derivative with copper, and blue and brown compounds with nickel and cobalt, respectively. Individual patterns can be secured by developing successive prints with alpha-benzoinoxime (copper, bright green), dimethylglyoxime (nickel, red), and alpha-nitroso-beta-naphthol (cobalt, brown). The iron pattern is made by stripping the surface with 20 per cent hydrochloric acid and washing the medium with 5 per cent ammonium hydroxide. The iron remains fixed as ferric hydroxide, and is separated from the bulk of the accompanying copper, nickel, and cobalt chlorides. The iron pattern is developed by immersing the print in a 2 per cent solution of potassium ferrocyanide containing about 0.1 per cent of hydrochloric acid which converts the ferric hydroxide into Prussian blue.

Figure 7. Sulfide Pattern of a Copper Ore

Specimen of chalcocite intergrown with bornite, from Butte, Montana. Area of pattern, full size. Exposure, 40 ma. for 10 seconds.

Since most sulfides are good conductors of the electric current, the electrographic technique is advantageously applied in the execution of the sulfide pattern of minerals.

The specimen is placed on a sheet of matte photographic printing paper (Eastman, Azo Grade A, No. 2), which has been moistened with a 1 per cent solution of sodium chloride and the mineral is connected to the negative pole of the battery. A current of 5 milliamperes per sq. cm. is allowed to flow for about 10 to 15 seconds, causing the migration of the negatively charged sulfide ion to the aluminum anode and its fixation as brown silver sulfide on the medium. Excess silver halides are then extracted by means of hypo and the print is finally washed in water (Figure 7). When the current is passed through the moist photographic paper the area acquires a pink tint, which is probably caused by the formation of reduction products of the silver halides. These subhalides dissolve in the hypo solution and do not cause any interference with the sulfide pattern. In applying the method to the localization of traces of sulfides, the exposure should not be prolonged beyond 1 minute, to avoid possible reduction of the emulsion to metallic silver. The brown colored pattern recorded on the paper is specific for sulfides in the absence of selenides and tellurides, which also react with the emulsion with the formation of black silver derivatives. The more commonly occurring arsenides and antimonides do not interfere and the method can be applied to the detection and localization of traces of sulfide segregations in minerals where arsenic and antimony predominate.

Table III. Classified Guide to the Literature on Analytical Patterns

C, contact print; E, electrographic print; R, autoradiographic print

Constituent	Mineral Sections	Metals and Alloys	Tissues	Plated Surfaces
Aluminum	..	..	..	E (*20*)
Antimony	C (*13*); E (*32, 41*)	E (*15*)	..	C (*62*)
Arsenic	C (*13*); E (*32, 41*); R (*27*)	..	..	..
Bismuth	C (*13*); E (*41*)	E (*15, 42*)	..	..
Cadmium	..	E (*15*)	..	E (*35*)
Chlorides	..	..	E (*67*)	E (*68*)
Chromium	..	E (*4, 23, 24, 35*)	..	E (*8, 69*)
Cobalt	C (*13, 30*); E (*39*)	C (*58*); E (*15, 44, 48*)	..	..
Copper	C (*13*); E (*39*)	C (*58*); E (*17, 44*)	..	E (*69*)
Gold	R (*27*)	..	..	E (*20, 69*)
Iodides	..	..	R (*34*)	..
Iron	C (*13, 30*); E (*41*)	E (*1, 17, 46*)	..	C (*54*)
Iron oxides	..	C (*56, 58*)	..	..
Lead	C (*13*); E (*41*)	E (*22, 35*)	R (*7, 53*)	..
Manganese	E (*29, 41*); R (*28*)	E (*15*)	..	..
Molybdenum	E (*41*)	..	..	..
Nickel	C (*13, 30*); E (*47, 49*)	E (*19, 25, 48*)	..	E (*23, 69*)
Nitrites	..	..	..	C (*43*)
Palladium	E (*29*)	E (*35*)	..	..
Phosphates	C (*52*); R (*27*)	..	R (*34*)	..
Phosphides	..	C (*37, 57, 58*); R (*66*)	..	..
Polonium	..	R (*9, 64*)	R (*51*)	R (*9*)
Potassium	R (*27*)	..	E (*67*)	..
Silver	E (*40*); C (*65*)	E (*17, 35*)	..	E (*69*)
Sodium	R (*27*)	..	E (*67*)	..
Sulfides	C (*12, 13, 30*)	C (*2, 5, 6, 37, 57*)	..	..
Thorium	R (*36*)	R (*63*)	..	..
Tin	..	E (*15*)	..	E (*20*)
Titanium	E (*3, 41*)	..	..	..
Tungsten	R (*27*)	..	..	..
Uranium	R (*36, 50*)	..	..	..
Zinc	E (*41*)	E (*15, 35*)	..	E (*35, 69*)

Further details of these techniques can be secured by consulting the original papers classified in Table III, which lists the methods applicable to the execution of analytical patterns of minerals, vital tissues, alloys, and electroplated surfaces. Most of these methods were developed during the past 10 years by a comparatively small number of workers. It is to be expected that with further developments in the synthesis of radioactive isotopes and with the discovery of new selective chemical reactions the general applicability of these pictorial methods of analysis will be greatly extended.

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Yeast Microbiological Methods for Determination of Vitamins

PYRIDOXINE

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THE discovery that pyridoxine belongs to the group of substances known as bios factors or yeast growth factors (*3*, *4*) was followed by attempts to use the effect in assay methods (*4*, *9*). Since the initial use of a yeast growth method for the assay of pyridoxine (*4*), certain improvements in the method have been made. The technique and apparatus have been simplified and the medium has been improved, but the principal change is the choice of a yeast strain especially selected for its specific response to pyridoxine. In a pyridoxine-free medium this yeast (culture 4228) grows very slightly, but if pyridoxine be added to the medium an extensive growth is observed, comparable to that observed in the presence of yeast water or malt extract.

Wherever possible the apparatus and materials required for the conduct of the assay have been chosen from the least expensive standard items available. The technique has recently been described in detail (*1*). Yeast suspensions, in selected 18-mm. Pyrex test tubes, are shaken in a Fisher-Kahn shaking apparatus at 30° C. Some workers (*9*) do not shake the yeast suspensions during the growth period but in the authors' hands this method has yielded irregular results. The extent of yeast growth after 16 and 18 hours is estimated by densitometric measurements, made directly on the tubes with the aid of a Lumetron No. 400 photoelectric colorimeter. A Mazda lamp provides the light, which is reduced in intensity by a gray glass-wire screen combination in place of a filter.

Solutions

Because the methods used in the determination of pyridoxine may also be employed for the determination of other vitamins—e. g., pantothenic acid, biotin, and inositol—it is convenient to prepare individual solutions of the various components of the basal medium and to combine them in the different ways required by the several methods.

Sugar and Salts Solution. One liter contains 200 grams of c. p. dextrose (anhydrous), 2.2 grams of monopotassium phosphate, 1.7 grams of potassium chloride, 0.5 gram of calcium chloride ($CaCl_2.2H_2O$), 0.5 gram of magnesium sulfate, 0.01 gram of ferric chloride, and 0.01 gram of manganese sulfate.

Potassium Citrate Buffer. One liter contains 100 grams of potassium citrate ($K_3C_6H_5O_7.H_2O$) and 20 grams of citric acid ($14H_3C_6H_5O_7.H_2O$).

Table I. Effect of Volume and Acid Concentration of Extraction Medium on Extraction of Pyridoxine from Dry Yeast

(100 mg. of 200 B dry yeast suspended in volume noted, heated at 20 pounds for 1 hour)

Volume	Concentration of Sulfuric Acid	Pyridoxine Determined
Ml.	*N*	*γ/g.*
10	0.055	31.0
50	0.055	37.0
100	0.055	37.0
180	0.055	39.3
180	0.0	27.2
180	0.028	38.4
180	0.111	37.4
180	0.222	35.7

Casein Hydrolyzate Solution, 80 ml. of S. M. A. Corp. "vitamin free" casein hydrolyzate (10 per cent solution) neutralized to pH 4.0 to 6.0 (alkacid paper), and diluted to 100-ml. volume.

Thiamine Solution, 10 micrograms per ml.

Inositol Solution, 1 mg. per ml.

Biotin Solution, S. M. A. Corp. biotin concentrate No. 5000, diluted so that it contains approximately 0.8 microgram per ml.

Calcium Pantothenate Solution, 200 micrograms per ml.

Yeast Inoculum

Culture 4228, a strain of *Saccharomyces carlsbergensis*, is carried on Difco malt agar slants. A slant is incubated for 24 hours at 30° and is then stored in the refrigerator for not more than 2 weeks. To prepare an inoculum for the assay a fresh slant is prepared 24 hours earlier and is also incubated at 30°. A quantity of fresh growth is removed by a sterile wire loop and suspended in 10 ml. of sterile 0.9 per cent saline in a colorimeter tube. The concentration of the yeast is estimated with the densitometer and is adjusted to a concentration of 1 mg. of moist yeast per ml. with additional sterile saline. The densitometer is conveniently calibrated with a suspension of moist compressed baker's yeast instead of culture 4228. With the authors' instrument a suspension of moist baker's yeast, 1 mg. per ml., shows a light absorption of 20 per cent. Five milliliters of the adjusted suspension are then added to 45 ml. of saline in an Erlenmeyer flask. The final suspension thus contains 0.1 mg. of moist yeast per ml. and is ready for use.

Preparation of Samples for Assay

Although pyridoxine is relatively soluble, it is extracted from most plant and animal tissues with difficulty. The Texas workers (*9*) have observed that autolysis increases the pyridoxine content of animal tissue extracts, but this technique is inapplicable to most foods and tissues. The authors have found that acid extraction increases the pyridoxine content of extracts as measured by the yeast method. The efficiency of the extraction depends upon the volume of the extraction medium as well as the presence of acid. This is demonstrated by the data of Table I. For most substances extraction with 180 ml. of 0.055 *N* sulfuric acid yields maximal values and this treatment has been used throughout this communication unless otherwise specified.

Insoluble materials should be powdered if dry, or macerated with water in a Waring blendor, or its equivalent, if infrangible. A portion containing between 2 and 4 micrograms of pyridoxine is suspended in 180 ml. of 0.055 *N* sulfuric acid—i. e., 1 ml. of 10 *N* acid and 179 ml. of water. The suspension or solution is heated in an autoclave at 9-kg. (20 pounds) pressure for 1 hour, then cooled, neutralized to pH 5.2, and diluted to 200-ml. volume. If the solution is turbid it is centrifuged and the clear supernatant liquid is used for the assay. In exceptional cases—e. g., white flour—the turbidity remains but can be removed if the extract is treated with a knife point of clarase at 37.5° for 30 minutes, followed by centrifugation.

Wheat and wheat products do not yield maximal pyridoxine values when treated as above, but require more acid. It has been found that 180 ml. of 0.44 *N* sulfuric acid—i. e., 8 ml. of 10 *N* sulfuric acid and 172 ml. of water—yield nearer maximal assay values with these substances.

The efficiency of the acid extraction of pyridoxine has been checked by comparison with enzymic digestion. Although the digestion with clarase does not always yield results as high as the acid extraction, the tendency is clearly toward the same values. Table II contains several instances where the two methods of extraction have been compared. Either clarase or polidase may be used, but the authors prefer clarase because it has less color and less pyridoxine. Their sample contained 1 microgram per gram. The enzymic digestion is performed as follows:

A portion of the sample containing between 2 and 4 micrograms of pyridoxine is suspended in a small quantity of water in a graduated test tube, 0.5 ml. of potassium citrate buffer (pH 5.2) is added, and the volume is made to 10 ml. The tube is steamed for 30 minutes, cooled, and a quantity of clarase equal to the weight of the sample, but not less than 100 mg., is added. A few drops of benzene are added, and the tube is tightly corked and incubated for 3 days at 37.5° or 2 days at 45°. At the end of the incubation the tube is steamed for 20 minutes and the contents are diluted to 200 ml.

The problem of obtaining pyridoxine extracts suitable for assay is not simply one of preparing a soluble form of pyridoxine; some soluble vitamin concentrates were found to contain a bound form of pyridoxine which was not active in the test until acid autoclaved or enzymically digested.

Method

Five milliliters of basal pyridoxine-free medium plus a solution of the unknown or of pure pyridoxine are placed in a series of test tubes together with water to make the total volume in each tube 9 ml. The tubes are plugged and steamed for 10 minutes,

Table II. Pyridoxine Content of Miscellaneous Substances

Description	Pyridoxine Determined	Pyridoxine Literature Values
	γ/ml.	*γ/g.*
Citrus		
Lemon juice (whole juice)	0.35	
Orange juice (whole juice) A	0.52	
Orange juice (whole juice) B	0.60	
	γ/g.	
Meat		
Pork liver (fresh)	5.9	3.3 (*8*), 1.7 (*9*)
Beef liver (fresh)	7.1	7.3 (*8*), 0.4 (*9*)
Pork muscle (fresh)	6.8	6.1 (*8*), 1.23 (*9*)
Beef muscle (fresh)	2.3	4.0 (*8*), 0.81 (*9*)
Liver concentrate powder, 1 to 20	41.8	45.0 (*2*)
	γ/ml.	*γ/ml.*
Milk		
Pasteurized (whole)		
A	0.56	1.3 (*8*), 1.7 (*6*)
B	0.51	
C	0.50	
D	0.60	
D[a]	0.50	
Evaporated (whole, not diluted)	0.62	
	γ/g.	*γ/g.*
Dry (skim milk)	5.5	
Wheat and wheat products		
Whole wheat[b]	4.8	4.6 (*7*), 7.6 (*6*)
Whole wheat[a]	4.7	
White flour (patent)[b]	1.2	2.2 (*7*)
Whole wheat bread (air dry)[b]	4.2	
White bread (air-dry)[b]	1.0	
	γ/day	
Urine		
Normal 24-hour excretion		
Subject A	143	
Subject B	128	
Subject C	127	
24-hour excretion following ingestion of 5.0 mg. of pyridoxine		
Subject A	241	
Subject B	284	
	γ/g.	
Yeast		
Baker's (fresh) A	6.9	
Baker's (fresh) B	4.9	
Baker's (fresh) C	9.1	
Brewer's (dry)	39.3	55 (*2*), 54 (*6*)
200 B-(dry)	40.0	
200 B-(dry)[a]	39.6	
Yeast extract (dry) A	120.0	
B	62.0	

[a] Clarase digestion, 3 days at 37.5° C. at pH 5.2.
[b] Extracted at higher acid concentration—i. e., 180 ml. of 0.44 *N* sulfuric acid, 1 hour at 20 pounds.

Table III. Typical Protocol

[To each tube are added 5 ml. of basal pyridoxine-free medium plus ingredients noted below. After sterilization 1 ml. of yeast suspension (0.1 mg. of moist yeast) is added to each. The tubes are then shaken at 30° for 16 hours, and the absorption measured, then returned to incubator for 2 hours and measured again.]

No.	H_2O	Added	16 Hours Absorption	16 Hours B_6	16 Hours B_6	18 Hours Absorption	18 Hours B_6	18 Hours B_6	Av.
	Ml.	*Ml.*	%	*mγ per tube*	*γ/g. or γ/ml.*	%	*mγ per tube*	*γ/g. or γ/ml.*	*γ/ml.*
1	4	0	17.5	..	.....	21	..	.....	.....
2	3.5	0.5 ≃ 5 *mγ* of B_6[a]	30	..	.....	35	..	.....	.....
3	3	1.0 ≃ 10 *mγ* of B_6	38.5	..	.....	44	..	.....	.....
4	2.5	1.5 ≃ 15 *mγ* of B_6	45	..	.....	50.5	..	.....	.....
5	2	2.0 ≃ 20 *mγ* of B_6	49	..	.....	57	..	.....	.....
6	1	3.0 ≃ 30 *mγ* of B_6	56.5	..	.....	64.5	..	.....	.....
7	0	4.0 ≃ 40 *mγ* of B_6	62.5	..	.....	68.5	..	.....	.....
8	3	1.0 ≃ 0.01 ml. of whole orange	29	4.5	0.45	35.5	5.5	0.55	
9	2	2.0 juice[b]	38.5	10.0	0.50	45	10.7	0.54	0.53
10	1	3.0	45	15.7	0.52	52.5	16	0.53	
11	0	4.0	51	22.3	0.55	58	21	0.53	
12	3	1.0 ≃ 0.01 ml. of approved	29.5	4.7	0.47	36	5.7	0.57	
13	2	2.0 pasteurized milk[c]	37.5	9.5	0.48	45	10.7	0.54	0.50
14	1	3.0	43.5	14.5	0.48	51	15	0.50	
15	0	4.0	48.5	19.5	0.49	56	19	0.48	
16	3	1.0 ≃ 0.1 ml. of urine, normal	34	7.2	0.072	40	8	0.08	
17	2	2.0 male[d]	45	15.7	0.078	51.5	15.3	0.077	0.075
18	1	3.0	51	22.2	0.074	59.5	22.5	0.075	
19	0	4.0	56	29	0.073	63	28	0.07	
									γ/g.
20	3.5	0.5 ≃ 0.05 mg. of yeast extract	32	6	120	37	6.3	126	
21	3	1.0 dry[e]	42	13	130	47	12	120	122
22	2	2.0	54	26	130	59	22	110	
23	1	3.0	61.5	39	130	66.5	34	113	
24	3.75	0.25 ≃ 0.125 mg. of liver pow-	30.5	5.2	41.6	35.5	5.5	44	
25	3.5	0.5 der 1 to 20[f]	38.5	10.2	40.8	44	10	40	41.8
26	3	1.0	51	23.2	44.4	56.5	20	40	
27	3.5	0.5 ≃ 0.25 mg. of dry 200 B	38.5	10.2	40.8	45	10.7	42.8	
28	3	1.0 yeast[g]	50.5	21.7	43.4	54.5	18	36	40.5
29	2	2.0	62.5	40	40	68	40	40	

[a] Pyridoxine hydrochloride. One millimicrogram (1 *mγ*) = 0.001 microgram. A solution containing 10 *mγ* per ml. may be preserved for at least a month without deterioration if acidified and protected from light. It may be protected from bacterial attack by benzene or by sterilization.

[b] 2 ml. of freshly prepared orange juice added to 177 ml. of water plus 1 ml. of 10 *N* H_2SO_4. Heated at 20 pounds for 1 hour, cooled, neutralized to pH 5.2, and diluted to 200-ml. volume.

[c] 2 ml. of whole milk treated as in *b*.

[d] 20 ml. of urine (normal subject); 24-hour excretion diluted to 2000 ml. volume, treated as in *b* except 159 ml. of water added.

[e] 100 mg. of dry soluble yeast extract dissolved in 179 ml. of water and treated as in *b* except final dilution to 1000-ml. volume.

[f] 100 mg. of liver concentrate powder 1 to 20 suspended in 179 ml. of water and treated as in *b*.

[g] 100 mg. of dry 200 B yeast suspended in 179 ml. of water and treated as in *b*.

cooled, and inoculated with 1 ml. each of the yeast inoculum. The tubes are then shaken at 30° for 16 to 18 hours and the yeast growth is estimated in the densitometer. A reference curve, which consists of a series of tubes containing 0, 5, 10, 15, 20, 30, and 40 millimicrograms of pyridoxine, is included in each assay run.

The basal medium contains the following ingredients for each 5 ml.: sugar and salts solution, 2.5 ml.; potassium citrate buffer, 0.5 ml.; casein hydrolyzate, 0.5 ml.; thiamine solution, 0.25 ml.; inositol solution, 0.25 ml.; biotin solution, 0.10 ml.; calcium pantothenate solution, 0.125 ml. For a set of 40 tubes, for example, forty times the above amounts are measured into a mixing cylinder and diluted to 200-ml. volume.

A typical assay run is given in Table III, which is the actual protocol of an assay in which representative materials were assayed. The results of the reference series (Figure 1) in per cent absorption are plotted on ordinary graph paper against millimicrograms of pyridoxine and the values for the unknowns obtained by interpolation. It is desirable to make readings at both 16 and 18 hours and to average all results except obvious slips, to obtain the estimated values. All results are reported on the basis of pyridoxine hydrochloride (Merck) employed as a reference standard.

Results

The number of pyridoxine assays by other methods which have been published is inadequate to provide a basis for critical comparison with the results of the yeast microbiological assay method. Table II contains the estimations made on a series of representative substances, compared with estimations which have been reported in the literature, obtained principally by the animal growth method of Conger and Elvehjem (*2*). The chemical method of Swaminathan (*6*) has provided a few results, but the chemical method of Scudi *et al.* (*5*) is apparently not sensitive enough for use with samples of ordinary potency.

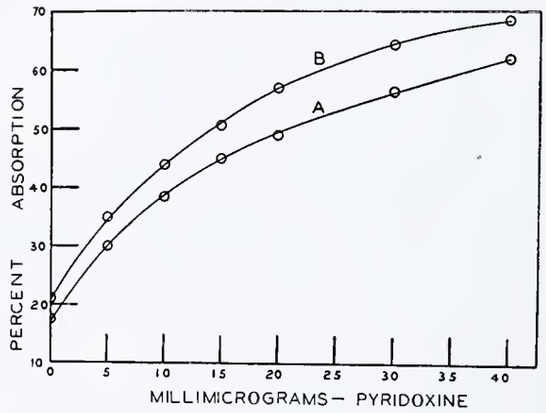

Figure 1. Reference Curve

A, 16 hours; *B*, 18 hours

Table IV. Recovery of Pyridoxine

Substance		Pyridoxine Content (by Assay)	Pyridoxine Added	Total Pyridoxine (by Assay)	Recovery
	Ml.	*γ/l.*	*γ*	*γ/l.*	%
Urine					
A	1000	60	100	153	93
B	1000	60	100	150	90
C	1000	159	100	283	124
D	1000	187	100	284	97
	Gram	*γ/g.*		*γ/g.*	
Yeast extract					
A	1	132	100	233	101
B	1	113	100	203	90
C	1	48	100	165	117
Dry yeast					
A	1	42	50	94	104
B	1	40	50	101	122
				Av.	104

The values obtained on fresh meat samples agree with the results of Waisman and Elvehjem (*8*). The Texas workers (*9*) report very low values for meats. In a more recent publication (*10*) the Texas group report assays on a number of foods, but apparently they have not changed their method of assay and the results are still very low.

The authors' values for fresh milk, about 0.5 microgram per gram, are significantly lower than the two values which have so far been reported. Stronger acid digestion and

clarase digestion both failed to give higher results. The analysis of dry skim milk is consistent with the fresh milk assays—i. e., about 11 times higher. The wheat assays are satisfactory except for the patent flour, which is lower than reported.

The assay of urine gave 135 micrograms per day as the average 24-hour excretion for 10 days by three normal male adult subjects. Ingestion of 5.0 mg. of pyridoxine was followed by an excess output of about 0.1 mg. during the next 24 hours. This is a rather low figure, but it may be estimated with reasonable precision and its smallness alone does not detract from its clinical significance.

The success of recovery experiments does not of itself establish the validity of an assay method, but it is a favorable sign. Table IV gives the results obtained when pyridoxine hydrochloride was added to a series of urines, yeast extracts, and dry yeasts. Each mixture was carried through the complete procedure. The recovery was calculated on the basis of the pyridoxine added and the average of 9 determinations was 104 per cent.

With regard to the specificity of the yeast microbiological method for pyridoxine determination, the following arguments can be adduced in favor of a specific response: The estimated values for the various extracts which have been tested show no perceptible change at different testing levels —i. e., no drift; no change is observed if the test is extended from 16 to 18 hours; acid digestion and enzymic digestion produce essentially the same extraction of the active principle; with a few exceptions the yeast method gives results which agree reasonably well with results obtained by the rat growth method; and added pyridoxine may be recovered from urine, yeast, and yeast extracts without appreciable loss or gain.

Summary

A microbiological method for the determination of pyridoxine employs a yeast strain (No. 4228) characterized by a specific response to pyridoxine. The yeast is grown in test tubes which are shaken at 30° for 16 to 18 hours. Yeast growth is estimated with the aid of a photoelectric colorimeter.

Recovery experiments are described and the pyridoxine content of a series of representative foods and other substances is reported.

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A Null-Reading Photoelectric Microdensitometer

For Use in Turbidimetry and Abridged Spectrophotometry

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The paper describes the details of construction and operation of a photoelectric densitometer, employing two photoelectric vacuum tubes in a balanced bridge circuit with electronic amplification. The circuit includes a voltage-regulator tube to counteract line voltage fluctuations. Only 1-ml. samples, in standard 75 × 10 mm. selected test tubes, are needed for all determinations. Readings are obtained by adjusting a light wedge, calibrated in per cent light transmission from 100 to 0, to balance the amount of light absorbed by the unknown over the control. The electronic circuit, operating on the null-reading principle, is used only to indicate the balance point. Once a calibration curve is obtained for any substance it can always be used for estimating the unknown sample, since the light wedge cannot change its calibration. That the data follow the Bouguer-Beer law can be seen from the linearity of the graphs obtained from a series of dilutions of India ink and copper sulfate, using the proper color filters. The instrument will be used in the measurement of the degree of turbidity resulting from mixtures of antigen and antibody in studies of precipitins.

IN ALL branches of biological research there is an urgent need for better methods of measurement, methods of greater speed, greater sensitivity, and greater reliability. To that end this paper is submitted, giving the details of construction, operation, and performance data of a new instrument for the measurement of colored and turbid solutions—viz., a photodensitometer, employing two photoelectric vacuum tubes in a balanced bridge circuit, with electronic amplification, in a stabilized circuit known to have great sensitivity and reliability.

This instrument is the culmination of a year's design, building, and testing by the writer in an attempt to find a better and more rapid method of measuring the turbidity of solutions. The writer (*2*) has been interested in a phase of serology, on the study of animal relationships, where the technique employed (*1*, *4*) involved measuring the amount of precipitate formed in the precipitin reaction between the interactions of antisera with homologous and heterologous antigens. The precipitate so obtained in the reaction has previously been measured by centrifugation at a known rate of speed for a fixed time in calibrated capillary tubes. While the results obtained have been statistically reliable, the time involved in their measurement has been a severe drawback. To this end it was determined to attempt to measure the

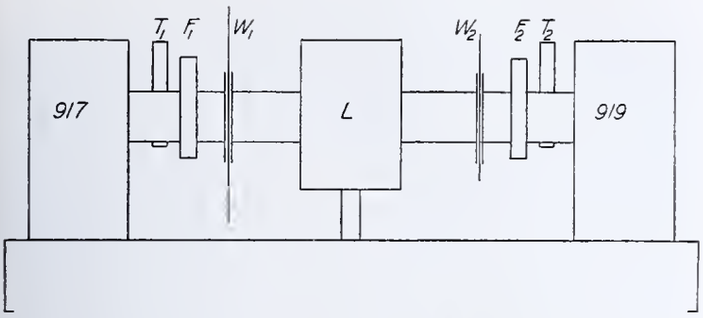

FIGURE 1. BASIC MECHANICAL ARRANGEMENT OF INSTRUMENT

T_1, T_2. Holders for test tubes containing samples to be compared. F_1, F_2. Filter compartments. W_1. Calibrated light wedge. W_2. Compensating light wedge for initial null setting. L. Ventilated light compartment for illuminating photocells found in compartments 917 and 919

amount of precipitate in suspension by means of photometry on the basis of the Bouguer-Beer law, applied to turbid systems (*8*). While the instrument was designed for this one specific purpose, it can be applied to many related problems as well as to the measurement of the density of colored solutions by the use of the proper color filters.

Instrument Types

Since the first report (*17*) of the application of the photoelectric principle to nephelometry and densitometry, many papers describing a number of instruments have appeared. These instruments fall into five general classes.

1. Many instruments (*6, 7, 10, 18, 19*) employ a sensitive galvanometer to measure directly the current output from a single self-generating barrier layer (photovoltaic) cell under variable conditions of light transmission. The difficulties are numerous. (*A*) Very sensitive and hence very delicate suspension galvanometers must be employed in the better instruments to measure the minute currents of the photovoltaic cell. (*B*) Such small currents can be amplified only with difficulty so that it is not practical to use an amplifier circuit in order to permit use of more rugged, less sensitive, and hence less delicate galvanometers. (*C*) The light source must be very constant; the ordinary 110-volt alternating current line supply is hardly adequate and the better instruments must therefore employ storage batteries. (The alternating current line voltage in the author's laboratory fluctuates steadily between 117 and 122 volts with frequent excursions to 115 and 125 volts. Even the best voltage-compensating transformers cannot maintain the necessary constant voltage required for illumination of the single photoelectric cell instruments, *16*.) This makes the instrument nonportable as well as requiring attention, frequent charging, and eventual replacement of the battery. (*D*) Individual photoelectric cells, while stated to be similar, will vary sufficiently to require new calibration curves in the event of their replacement. (*E*) Deterioration of photoelectric cells will require that calibration curves be constantly checked for reliability of the data.
2. A few instruments (*17, 21*) have been constructed which use a single photoelectric vacuum tube and a direct reading galvanometer. The difficulties listed above still apply to these vacuum tube instruments.
3. Some instruments (*5, 13, 15, 20*) employ a single photoelectric vacuum tube with an electronic amplifier system where essentially the photoelectric cell electronic emission is amplified so that sensitivity can be increased and more rugged meters (usually a milliammeter) employed to measure the current output. However, objections *C*, *D*, and *E* still apply to these vacuum tube instruments.
4. Some instruments (*3, 9, 23*) employ two photovoltaic cells in a bridge circuit using a galvanometer to determine the null point and a calibrated potentiometer for obtaining readings in a linear scale of the percentage of light transmitted through the solution on a logarithmic scale for a direct reading of density. While these instruments compensate for line voltage fluctuation and photocell deterioration, they are limited in their sensitivity by the circuit employed and by the galvanometer sensitivity.
5. Other instruments (*11, 22*) use photoelectric vacuum tubes in a bridge circuit with methods of reading the transmission of light or the density as in the previous type. These instruments can use an electronic amplifier system (*12, 14*) to increase the sensitivity to a degree limited only by the amplifier system employed. The instrument described in the present paper is of this type, employing a stabilized amplifier system.

The instruments described in this brief review by no means cover the entire field, but are mentioned only to describe the essential types used by various workers to study many problems: analytical chemistry, routine clinical analyses, spectrophotometry, etc. A very complete list of contributors to this field is found in the literature (*8, 14*).

Basic Design of the Instrument[1]

The instrument described in the present paper attempts to improve further upon the use of photoelectric cells in turbidimetry and abridged spectrophotometry.

It employs two RCA photoelectric vacuum tubes in a "null" circuit with one stage of vacuum tube amplification. Since both photocells are illuminated by the same light source, changes in its intensity will have the same effect on both photoelectric cells, but no effect on the null reading. The amplifier circuit, as well as the photoelectric cells, is voltage-regulated by means of a VR 105–30 voltage regulator tube, so that changes in line voltage have a negligible effect upon the circuit. All vacuum tubes are working at minimum potential for long life and stability. Inherent in the design of the instrument is the principle that a very small change in the light intensity falling on one photoelectric cell over that falling upon the other cell, after they had been previously adjusted for the null point, causes a change in the electronic emission of one photoelectric cell over that of the other, so that the grid of the electronic amplifier

[1] Since this paper was accepted for publication improvements in design include (1) an interchangeable condensing lens system to increase sensitivity when using certain filter combinations, (2) the addition of RCA 926 photoelectric vacuum tubes, with greater sensitivity in the blue region of the visible spectrum, and (3) adaptations for use of square test tubes of precision manufacture (6 × 6 mm. in inside diameter and 75 mm. in length).

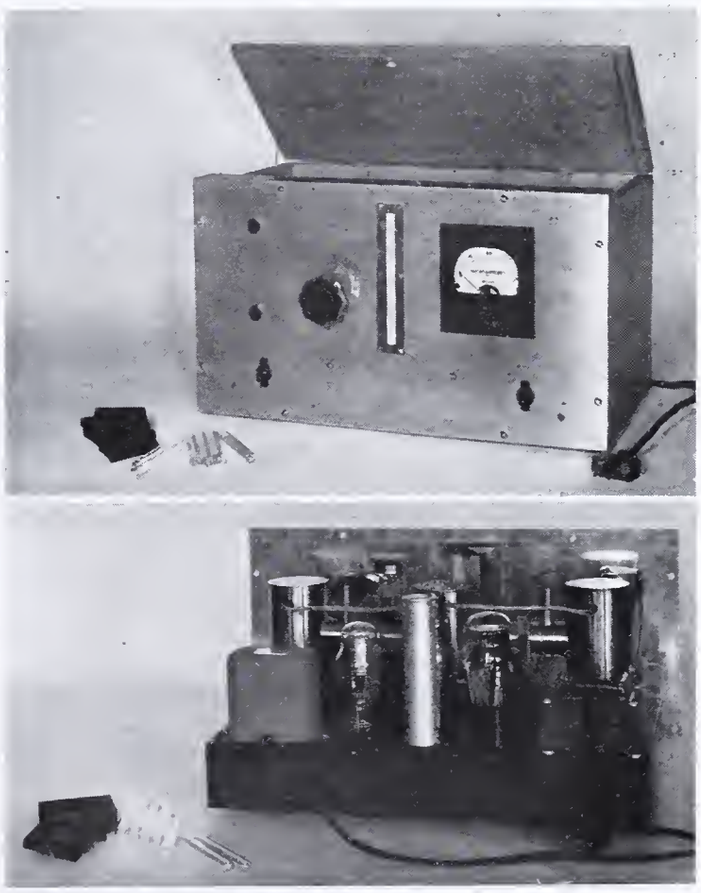

FIGURE 2. PHOTOGRAPH OF DENSITOMETER

Above. Cover open ready for use
Below. Instrument as seen from back with cabinet removed

becomes either more or less negative than it was when at the null point. This in turn causes the microammeter in the plate circuit to register either zero or full-scale deflection. A light wedge is then adjusted to cause the meter to register the null reading again, bringing the photoelectric cells back to the same degrees of illumination and electron emission as originally. The amount of movement of the wedge required to bring the instrument back to the null point is a measure of the amount of light absorbed by the unknown sample. The instrument is thus adjusted to an arbitrary null point, for each reading, by regulating the light which falls upon the two photoelectric cells.

Two similar sample tubes when placed successively in the path of light going to one photoelectric cell will not cause a change in the null point, while if one of the tubes is replaced by an unknown tube of differing density, less light will pass through to fall on the one photoelectric tube and the calibrated light wedge will have to be adjusted to return the instrument to the null reading. Graphs are plotted, on semilogarithmic paper, of the wedge readings obtained from solutions of known concentration, and the unknown sample concentrations are obtained by reference to the known graphic readings. Once a calibration curve has been obtained for any substance, it can always be used for estimating the unknown sample, since the light wedge cannot change its calibration, as is possible using direct readings from photocellmeter instruments. The degree of sensitivity of the instrument to minute differences in light intensity can be regulated by varying the voltages applied to the photoelectric cells, or to the plate and screen of the electronic amplifier tube. Various degrees of sensitivity are incorporated in the instrument by varying the screen voltage with preset resistor clips and a multipole switch.

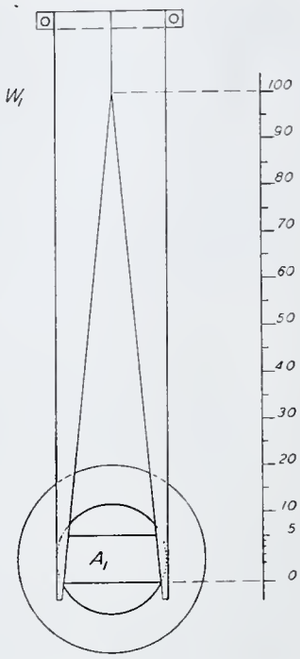

FIGURE 3. DIAGRAMMATIC VIEW OF LIGHT-DECREASING WEDGE AND ASSOCIATED CALIBRATED SCALE

A_1. Trapezoidal window area admitting light to filter, sample, and then to photocell 917. Area of trapezoid = $^1/_2\ (a + b)\ h$ where base, a, = 21 x, top, b, = 19 x and altitude, h, = 10 x. 10 x = $^3/_8$ inch. Lowering of wedge from scale reading of 100 to 90 decreases light window area 20 square units, or 10 per cent of total, etc. Window, wedge, and scale are constructed with precision.

Mechanical Design

Figures 1 and 2 illustrate the basic design of the instrument. The housings for the lamp, L, and for the two photocells, 917 and 919, are constructed of brass sheeting and tubing and rigidly supported on a steel chassis. The horizontal tubes of smaller diameter, connecting these three upright compartments, carry the light through the tapered light wedges, W_1 and W_2, through the filter chambers, F_1 and F_2, and in turn through the test tube holders, T_1 and T_2, to the photocell chambers. These chambers are all blackened inside and light-tight. Diffusion disks of flashed opal glass are used for even illumination of the light-adjusting wedges and so that the entire cathode area of the photocells will be utilized. The test tube holders of round brass tubing are of suitable size for 10 × 75 mm. serological test tubes and have milled vertical slots 3 by 10 mm. on each side for the transmission of light through the sample (1-ml. samples are sufficient for all analyses). The filter chambers are 0.93 cm. ($^3/_8$ inch) in width to allow for several 3.1-cm. ($1^1/_4$-inch) filter circles in combination for limiting the frequency range of light transmission. The filters are assembled in hard-rubber holders for easy replacement.

W_2 is the light-compensating wedge for balancing differences in light transmission of different filters and for the initial null setting. It is adjusted by means of a vertical screw and cam. The calibrated wedge, W_1, with its associated scale graduated in per cent of light transmission is illustrated diagrammatically from an end view in Figure 3. It is similar to W_2 except for its precision manufacture. This wedge is raised and lowered with a rack and pinion whose shaft projects through the aluminum panel for manual control. The calibrated scale is also located on the panel for easy reading and consists of a translucent scale upon which is projected a shadow of a fine wire illuminated by the narrow beam of a pen-light flashlight bulb, indicating the position of the wedge. The scale is calibrated in increments of 0.5 from 100 to 0 in terms of the per cent of light transmitted through the trapezoidal window, A_1, from fully opened to fully closed. Lowering of the wedge each 0.93 cm. ($^3/_8$ inch) decreases the window area 10 per cent and is linear except for the last 5 per cent, as indicated in Figure 3. A_1, W_1, and the scale have been constructed with mechanical and mathematical exactness.

Also mounted on the chassis are the amplifier tube, voltage regulator tube, rectifier tube, filter condenser, choke coil, and transformers. Under the chassis are the resistors, with all the wiring thus concealed from view. All critical circuits of the photoelectric cells and amplifier tube are shielded to eliminate any stray capacitance effect by the operator.

Photoelectric Vacuum Tube and Amplifier Circuit

The circuit used in employing the two photoelectric vacuum tubes with their amplifier is shown in Figure 4. The rectifier and filter circuit supplies the high voltage to the voltage divider, $R_1\ R_2\ R_3$. The voltage across the resistor, $R_2\ R_3$, is maintained at 105 volts by the voltage regulator tube, VR 105-30. The suppressor grid of the 38 amplifier tube and the cathode are grounded. The resistor, R_3, is below ground potential, so that the cathode of photoelectric cell 917 is adjusted for 22 volts below ground potential, while the anode of photoelectric cell 919 is operated almost an equal potential above ground. The 919 cathode and the 917 anode lead to the 38 grid and are essentially at ground potential when the photocells are equally illuminated or at zero illumination. The plate of the 38 amplifier is set at a low potential (25 volts) for stability and constancy of operation of the amplifier. The screen is set at a sufficient positive potential for the desired sensitivity and is variable in several steps (5, 10, and 15 volts).

A resistor in series with the meter and plate will prevent excessive plate current flow for the protection of the microammeter. Operating the circuit in this fashion with low plate, screen, and photoelectric cell potentials ensures stability and constancy of vacuum tube characteristics, even though extreme sensitivity is forsaken. However, the sensitivity which the instrument exhibits is much more than even the best of mechanical construction and experimental error warrant. If still greater sensitivity were to be desired and could be used, all voltages could be proportionately increased. The writer has

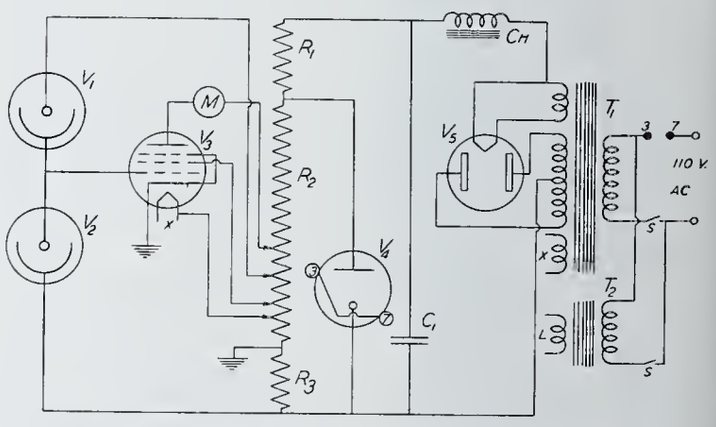

FIGURE 4. BALANCED CIRCUIT WITH HIGH DIFFERENTIAL SENSITIVITY AND STABILIZED HIGH-VOLTAGE SOURCE

V_1 = RCA 919; V_2 = RCA 917; V_3 = 38; V_4 = VR 105-30; V_5 = 80.

R_1 = 7000 ohms, 25 watts; R_2 = 7500 ohms, 75 watts; R_3 = 2000 ohms, 25 watts.

M = 0–200 microammeter, Weston, Model 301; C_1 = 16-mfd. electrolytic condenser (negative terminal insulated from ground); T_1 = power transformer (Thordarson T13R11).

Filament winding to 38 amplifier reduced from 6.3 to 4.0 volts by series resistor; T_2 = 115-5V step-down transformer (U. T. C. S-54) for photocell illuminating lamp; S = switches inserted in primaries of both transformers T_1 and T_2.

Contact pins 3 and 7 of VR 105-30 are inserted in A. C. power input lead as a safety measure; Ch = choke (Thordarson T68Co8).

Power supply indicating pilot light and dial illuminating lamp with appropriate resistors are not shown. Microammeter is protected by 150,000-ohm series resistor to limit current flow to slightly less than full scale. Heater of 38 amplifier is tied back approximately 9 volts above ground.

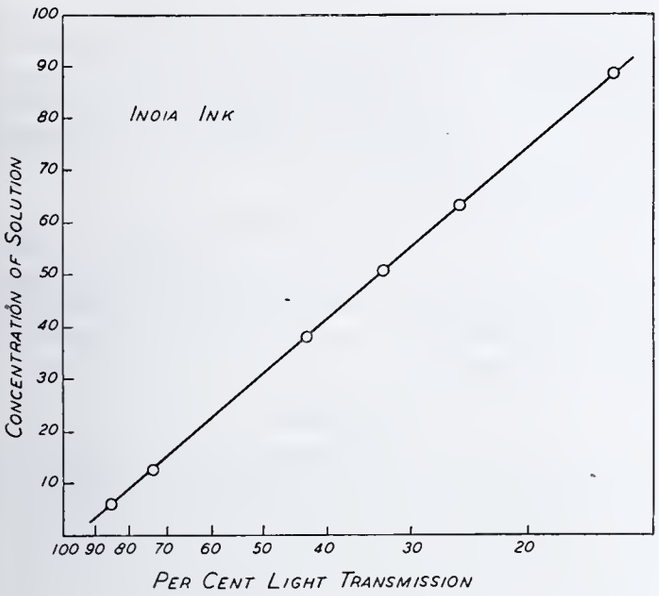

Figure 5. India Ink Calibration Curve

Concentrations of samples tested are in terms of per cent concentration of arbitrary India ink standard dilution (sample 2) taken as 100 per cent.

experimented with possible variations and has found that the instrument has much more sensitivity to differences in light intensity upon the two photoelectric cells than is needed.

The electrical circuit functions on the principle that if more light falls on one photoelectric cell (917) than on the other (919), after the circuit has previously been balanced for an arbitrary null point, the microammeter will deflect to zero, indicating that the grid of the 38 amplifier tube has become temporarily more negative as a result of greater electron flow from the cathode to the anode of the 917 photocell. To return the circuit to balance at the null point, wedge W_1 must be adjusted to reduce the amount of light falling upon photocell 917. The amount of movement of wedge W_1 indicates by its calibrated scale the area of the window which was removed to cause the proper decrease in light transmission to the 917 to compensate for the amount of light absorbed by the unknown solution, over the control, when placed in the path of light going to photoelectric cell 919.

Operation of the Instrument

The light from L (Figure 1) is allowed to fall upon both photoelectric cells (917 and 919). In the light path to each of these photoelectric cells is placed one of the two identical control samples, T_1 and T_2 (usually distilled water). With the calibrated wedge, W_1, set at 100 for full light transmission, wedge W_2 is adjusted so as to cause the microammeter to register the arbitrary null reading (100 microamperes). Then sample T_2 is removed and a solution of some substance of unknown concentration is inserted in its place. If more light is absorbed by this unknown tube (less transmitted) than was absorbed in sample tube T_2, W_1 is moved down so as to decrease the amount of light passing through T_1 until the same arbitrary null reading is again obtained.

The reading from the calibrated scale of W_1 is taken as showing the amount of light transmitted by the unknown tube compared with the control sample. Its concentration is obtained by referring to the graph obtained previously by plotting the light transmission of a series of tubes of increasing but known concentrations of the same substance prepared in the same way. Readings are made only on the basis of the amount of movement of the wedge required to compensate for the amount of light absorbed by the unknown sample tube over the amount absorbed by the standard comparison tube, which is a fixed or constant amount for all densitometric studies. The two photoelectric tubes in the null circuit are used only to determine to what position W_1 must be moved to again obtain a balance in the circuit.

Since the mechanical setup of the apparatus is stable under all conditions, the instrument does not depend upon the photoelectric cells for anything but an indication of the light balance point for the null reading. In fact, even though the photoelectric vacuum tubes are replaced by tubes of different sensitivities, the instrument can be adjusted for the same null reading by mechanically adjusting the null setting wedge, W_2. Replacement of the amplifier, voltage regulator, and rectifier tubes causes but minute and insignificant deflections in the null point of the instrument, which can easily be reset.

Table I. Reliability of Instrument

[In being able to duplicate data using a dilute India ink solution (sample 1) as a standard of 100% concentration]

Concn. of Sample	Light Transmission						
	7-1-42	7-1-42	7-1-42	7-1-42	7-1-42	7-2-42	7-7-42
% of standard	%	%	%	%	%	%	%
0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
1	96.0	96.1	96.3	96.1	96.2	96.0	96.0
2	91.7	91.7	92.0	91.7	91.7	91.6	91.4
5	80.7	80.4	80.4	80.5	80.5	79.6	80.4
10	65.0	65.3	65.3	65.3	65.0	64.2	65.1
25	38.2	38.5	38.4	38.6	38.4	38.0	38.5
50	17.0	17.3	17.3	17.3	17.2	17.4	17.2
75	7.3	7.5	7.6	7.5	7.5	7.0	7.4
100	3.1	3.5	3.4	3.5	3.4	3.2	3.4

Performance Data

For testing the reliability of the instrument in turbidity studies, readings were made using various dilutions of an arbitrary India ink standard (sample 1). The data obtained without the use of filters are illustrated in Table I and show the reliability of the instrument in being able to duplicate the readings over a period of days. The results are very consistent, even though from day to day differences are to be expected in line voltage fluctuations and in duration of use of the instrument. Figure 5 illustrates the data obtained in a similar study from a different India ink standard (sample 2) but using essentially monochromatic light by means of Corning No. 396 and Wratten No. 74 filters. That the readings follow the Bouguer-Beer law is shown by the linearity of the data as plotted.

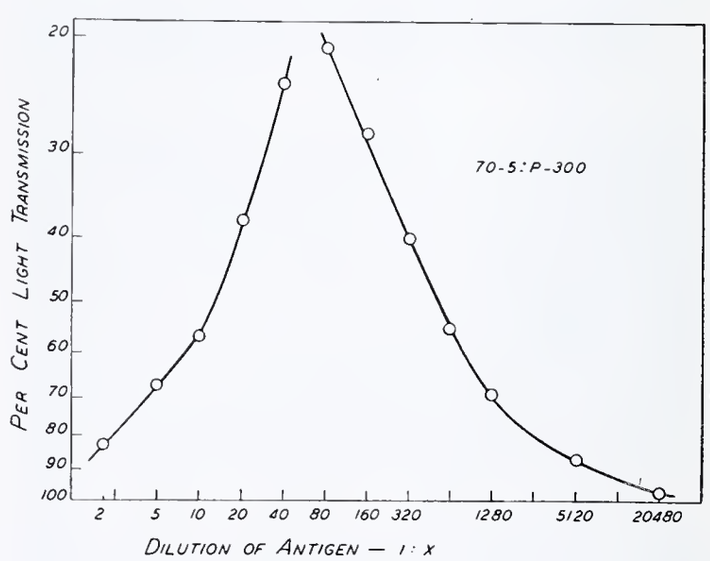

Figure 6. Degree of Turbidity

Expressed in per cent light transmission resulting from 0.5 ml. of anti-pig rabbit serum reacted against 0.5 ml. of increasing dilutions (decreasing concentrations) of its homologous pig serum antigen.

Figure 6 shows the results of a serological study using a constant dilution of anti-pig rabbit serum (70-5) reacted against serial dilutions of its homologous pig serum antigen (P-300). The graphical data illustrated here are typical and correspond with the volumetric studies of Baier (*1*). They also illustrate the application of the instrument to studies of serological relationship (*2*).

Since the photoelectric cells employed in the instrument are very sensitive in the red and infrared (greatest sensitivity at 800 millimicrons), readings were obtained against dilutions

of a standard solution of copper sulfate, which contained approximately 6 per cent (6.1336 grams of cupric sulfate pentahydrate per 100 ml. of water) of copper sulfate. Wratten No. 70 filters were used in obtaining the data illustrated in Figure 7.

Discussion

The need of a reliable and rapid method for the determination of turbidity in 1-ml. samples led to the development of the present instrument. The various instruments available were unsatisfactory, chiefly because they required larger samples for analysis. At the same time it was obvious that storage and B-batteries are bulky and troublesome, so that there was a need for a circuit incorporating voltage regulation in order to use the usual 110-volt alternating current source. Furthermore, individual photoelectric cells vary in sensitivity and linearity, so that a bridge circuit with a mechanical mechanism to measure per cent light transmission would minimize these possible sources of error.

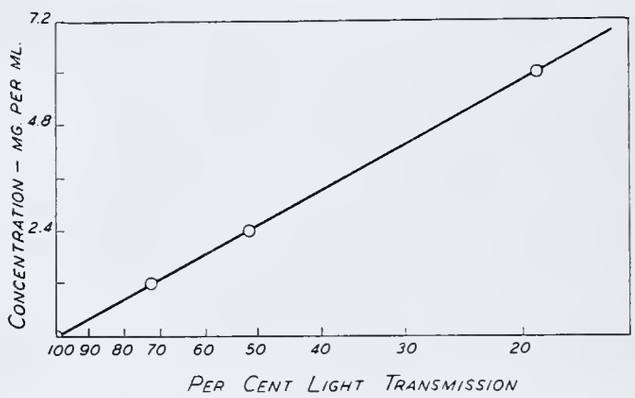

FIGURE 7. CALIBRATION CURVE FOR COPPER SULFATE

Dilutions made from standard copper sulfate solution containing 6.1336 grams of $CuSO_4.5H_2O$ in 100 ml. of H_2O.

With the exception of the instrument of McFarlan, Reddie, and Merrill (*12*) the photoelectric microdensitometer described here is the only one the author knows which uses an amplifier in conjunction with photoelectric vacuum tubes in a null circuit where readings are obtained from a calibrated light wedge. However, their instrument used an alternating current amplifier without voltage regulation while the instrument described in the present paper has a direct current amplifier with all high voltages stabilized against line voltage fluctuations. Müller (*14*) stated that a circuit of this type has high differential sensitivity and that "the trend in design of photoelectric photometers will be in the direction of vacuum cell–amplifier combinations of extreme sensitivity coupled with associated circuits for regulation, automatic compensation, and stability maintenance." He suggests a circuit having such possibilities, very much like the one utilized in the present instrument.

From the graph and table presented for India ink it can be seen that the data essentially illustrate the author's contention that the instrument shows great sensitivity and reliability. Within the limits studied in the dilutions the graphs are straight lines, following closely the theoretical aspects of the Bouguer-Beer law, with an inverse logarithmic relationship between light transmission and concentration.

Since in turbidity studies this linearity is shown between concentration and per cent light transmission, the instrument will be used in determining the degree of turbidity resulting from the reaction of an antiserum against varying dilutions of homologous and heterologous antigens. Heterologous turbidity measurements will be expressed in terms of per cent of the homologous turbidity measurement taken as 100 per cent. While the data must be plotted semilogarithmically, turbidity comparisons or relationship values in per cent must be determined by arithmetically determining the areas under the curves or by an arithmetic summation of the ordinates of the points which make up the curve using any uniform scale, not the logarithmic scale.

For the application of the instrument in "colorimetry" using the proper color filters, graphs are plotted, on semilogarithmic paper, of the wedge readings in per cent light transmission obtained from solutions of known concentration, and the unknown sample concentrations are obtained by reference to the graph.

Summary

Details of construction and operation are given for a photoelectric densitometer, which employs a balanced photocell circuit with electronic amplification operating on the null reading principle. The instrument is operated entirely from the usual 110-volt alternating current supply.

The apparatus includes a regulated high-voltage source unaffected by variation in the usual line voltage.

Readings in terms of per cent transmission of light are obtained by means of a calibrated wedge decreasing the size of a light-transmitting window.

The photocells are used only to indicate balance in the circuit, not to indicate per cent light transmission. Only 1 ml. of a sample is needed and selected 10 × 75 mm. serological test tubes are used.

Data are given to show its use as a turbidimeter and abridged spectrophotometer.

Acknowledgment

The writer wishes to acknowledge with appreciation the invaluable aid in the circuit design and the many helpful suggestions freely given by Hans U. Hjermstad, vice president of the Federal Electric Company of Chicago. He is also indebted to the staff of the Physics Department of the University of Wisconsin in Milwaukee for their many suggestions and for the use of the machine shop facilities.

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Semimicrodetermination of Chlorine, Bromine, and Iodine in Organic Compounds

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THE analytical procedure presented below for determination of chlorine, bromine, and iodine in organic compounds makes use of the Parr sodium peroxide semimicrobomb described previously (*12*). Operations are on a scale intermediate between that of the familiar macromethod (*8, 11*) and that of the micromethod of Elek and Hill (*2, 6*)—i. e., adapted to analysis of samples of semimicro size (20 to 50 mg.). The determination is gravimetric.

The Volhard volumetric procedure, when applied following decompositions in the sodium peroxide semimicrobomb, encounters certain difficulties which decrease the accuracy of this usually excellent method. The conditions of volume and salt concentration make necessary a solution of thiocyanate not weaker than 0.02 *N* if end points are to be satisfactory. With thiocyanate solution of this strength, and with semimicrosamples, the effective titrations are relatively small (especially with bromine and iodine), and the reproducibility of results is thus impaired. The alternative of increasing the samples considerably, so as to obtain larger effective titrations, was judged to be undesirable, as it would involve some danger of approaching or exceeding the capacity of the oxidizing charge, and would increase the consumption of sample material in some cases to that of the macromethod.

Direct titration of halide ion by silver nitrate with the aid of an adsorption indicator—e. g., dichlorofluorescein (*7, 10*)—was not submitted to trial, as the relatively high concentration of electrolytes unavoidably present constitutes a possible interference (*7*; cf. *1*).

Following decompositions of samples in the sodium peroxide bomb bromine is present partly as bromate [Beamish (*2*) reported bromate to be formed in a steel bomb but not in a nickel bomb], and iodine is present almost wholly as iodate. When a volumetric determination is intended it is ordinarily satisfactory to add an excess of standard silver nitrate solution to the alkaline extract, then to acidify, add hydrazine sulfate to reduce bromate or iodate, and finally to titrate excess of silver ion (*8*). When a gravimetric determination of halogen is intended it is necessary to filter the liquid before addition of silver nitrate, as the alkaline extract of the bomb contents contains undissolved particles. It is permissible to filter the alkaline extract through asbestos in a Gooch crucible, provided any dark residue on the filler is extracted with dilute nitric acid. Such residue may consist of, or contain, some metallic compound contributed by the bomb itself, and in one series of trials (see results for *o*-iodobenzoic acid in Table I) indications were clear that the residue contained part of the halogen.

In the gravimetric determination of chlorine a preliminary filtration of the alkaline extract is unnecessary, as the absence of chlorate makes it possible to acidify the solution, filter, and then add silver nitrate. In the gravimetric determination of bromine or iodine it is essential to add either the silver nitrate or the reducing agent before the solution is acidified. The addition of silver nitrate to the filtered alkaline solution, as in the volumetric procedure (*8*) led to irregularity in the determination of bromine, though it was satisfactory in the determination of iodine. Results for bromine were too high, and the precipitate was discolored after acidification of the liquid, suggesting contamination of the precipitate; the use of nitric acid in excess only partially eliminated this effect. In the determination of iodine no similar interference was observed, but in this case the initial precipitate of silver iodide was small, most of the iodine (present as iodate) being precipitated later from acid solution, so that contamination of the precipitate in the alkaline solution was slight. In order to have available if possible a single procedure useful for the gravimetric determination of both bromine and iodine, the feasibility of reducing bromate and iodate in the alkaline liquid was investigated. It was found that the small amount of bromate usually present is readily reduced by hydrazine in the hot alkaline solution, and that the relatively larger amount of iodate is completely reduced if the heating period is somewhat extended. To complete the analysis the solution is acidified and filtered, and the halide is precipitated by silver nitrate.

The use of glass ampoules for liquid samples introduces difficulties which are not readily overcome, and which are absent if gelatin capsules are used. In the sodium peroxide bomb a glass ampoule undergoes a silica fusion of its thinner parts (*13*), and later acidification of the liquid may cause precipitation of gelatinous silicic acid which presents an obstruction to the convenient gravimetric determination of halide. An earlier study of this difficulty (*17*), as it affects the macroprocedure, showed that satisfactory volumetric determination of halogen is possible by the Volhard titration properly executed (*9, 15*) in presence of the silicic acid and silver halide. Attempts to use a similar procedure in the semimicromethod confirmed the relatively low precision of the Volhard semimicrotitration as here applied, and revealed also that glass ampoules, if not broken before or during the mixing of the charge, may protect samples sufficiently to be a cause of incomplete decompositions. Acceptable results were obtained for chlorine in substances readily decomposable or not highly volatile (*o*-chloroaniline, *o*-chlorobenzoic acid; the latter decomposed in presence of, but not in, a glass ampoule). With substances relatively resistant or volatile (chlorobenzene, bromobenzene, *n*-butyl chloride) results were variable and not satisfactory.

It was found that liquid samples, if not too resistant or too volatile, can be decomposed satisfactorily if introduced in gelatin capsules, as suggested by Pflaum and Wenzke for the decomposition of fuming or deliquescent fluorine compounds in the sodium peroxide macrobomb (*14*). Trials with small (5 × 15 mm.) gelatin capsules showed complete destruction of the capsules, no increase in the blanks, and satisfactory gravimetric results. The use of gelatin capsules apparently introduces no complication; the analysis may be completed as for solid substances. Results by this procedure, which is the one recommended for liquid substances, are given in Table I.

The applicability of the sodium peroxide bomb method, on any scale, to the analysis of liquids appears to be limited. A considerable experience with the macro- and semimicromethods in this laboratory does not encourage great confidence in the certainty of complete decompositions of liquids which are resistant to decomposition or even moderately volatile, if samples are introduced enclosed in vessels (ampoules or capsules) which may afford a certain amount of

initial protection. Volatile liquids may be handled with reduced danger of loss, and may be decomposable with increased certainty, if a little anhydrous alcohol (or other solvent which is halogen-free and not too volatile or resistant) is introduced into the gelatin capsule before the sample is added and weighed (*16*). Light or readily electrified solids may not be properly decomposable in the sodium peroxide bomb. Specimens of halogenated fluoresceins, which may be very susceptible to frictional electrification, could not be satisfactorily analyzed by the macroprocedure. Even when an overlying layer of sodium peroxide was added to the previously well mixed charge visible quantities of undecomposed sample were present on the internal surface of the cover after decomposition, the alkaline extract showed fluorescence, and the results were variable and too low.

Procedure for Gravimetric Semimicrodetermination of Chlorine, Bromine, and Iodine

The Parr semimicrobomb required was described in a previous paper (*12*). Gooch crucibles, of 10-ml. capacity, should be prepared for use in a manner analogous to that described in the procedure for determination of sulfur (*12*), and should be brought to "constant" weight (within 0.03 mg.). Gelatin capsules, required for liquid samples, can be secured from drug supply houses. Those used were approximately 5 mm. in diameter and 15 mm. in length, and weighed about 0.04 gram.

Solid Substances. Transfer to the bomb cup 0.2 gram of powdered potassium nitrate (cf. *3*). Introduce the finely powdered (cf. *3*) sample, which should be of size to yield about 20 to 50 mg. of silver halide, and should be weighed with an accuracy of 0.02 mg. Add powdered sucrose (starch, lactose, benzoic acid) in such amount that the total weight of sample and other carbonaceous material is 0.2 gram. Weigh rapidly 4 grams (±0.1 gram) of granular sodium peroxide (low in chlorine) and transfer it to the cup. A satisfactory procedure, especially in humid weather, is to support the bomb cup on the left-hand pan of a trip scale, place a small beaker on the right-hand pan, and introduce water to counterpoise the bomb cup, into which the sodium peroxide may then be weighed directly and rapidly. A measuring cup of suitable capacity would be convenient and sufficiently accurate. Make certain that the gasket is in good condition, and then adjust the bomb cover in place and secure it firmly by tightening the screw collars. To mix the charge, shake the apparatus vigorously, and finally tap the bottom of the cup against the table top to settle the charge.

Liquid Substances. Transfer to the bomb cup 0.2 gram of powdered potassium nitrate, 0.16 gram of sucrose, and 4.0 grams of granular sodium peroxide. Fasten the cover in place, and thoroughly mix the charge by shaking. Weigh the sample (to yield 20 to 50 mg. of silver halide) in a gelatin capsule. Remove the cover of the sodium peroxide bomb, and thrust the capsule and the contained sample into the center of the previously mixed charge, so that the capsule is completely covered. Fasten the cover in place, but do not attempt further mixing of the bomb contents.

Decomposition. Support the apparatus in a vertical position by means of a clamp and ring stand, and at such height that the cup can be heated by the flame of a blast lamp seated on the table top. For the present, and until the explosion hazard of this apparently safe apparatus is better known, it is advisable to wear goggles and to place a safety glass screen, or an iron plate, in front of the bomb while the charge is ignited. Adjust the blast flame so that it is 7.5 cm. in length and 3 to 4 mm. in diameter. First bring the tip of the flame to a point about 1 cm. below the bomb for about 15 seconds, then move the flame so that the tip touches the bottom of the cup, and continue heating thus for 30 seconds more. A longer or more severe heating may melt the gasket.

Analysis. Cool the bomb, finally in tap water, and remove the cover. Wash the lower surface of the cover with a fine stream of hot water, collecting the washings in a 125-ml. beaker. Wash the cup externally (discard these washings) and then, with the aid of a thick glass rod, place the cup on its side in the beaker. Add water to a volume of 25 ml., cover the beaker, and warm gently to dissolve the fused mass. Using the glass rod, lift the bomb cup above the liquid and wash the cup externally with a fine stream from the wash bottle. Then grasp the cup between the fingers and wash the interior, collecting the washings with the main extract. Cover the beaker and heat the liquid until gas evolution practically ceases, indicating decomposition of

Table I. Determination of Chlorine, Bromine, and Iodine in Organic Compounds

(Following decomposition in the sodium peroxide semimicrobomb gravimetric procedure)

Compound	Sample	Silver Halide	Halogen Found	Halogen Calculated
	Mg.[a]	*Mg.*[a,b]	%	%
p-Aminophenol hydrochloride	23.78	23.36	24.30	
	24.69	24.26	24.31	
	34.38	33.46	24.08	
	38.85	38.36	24.43	
			Av. 24.28	24.36
p-Nitrochlorobenzene	30.35	27.40	22.33	
	45.59	41.18	22.39	
			Av. 22.36	22.51
o-Chlorobenzoic acid	39.94	36.31	22.49	
	33.65	30.63	22.52	
	39.96	36.03	22.30	
	24.67	22.49	22.55	
	24.65	22.41	22.49	
	23.40	21.24	22.45	
	19.57	18.09	22.87	
			Av. 22.52	22.65
Methylene-*p*-chloroaniline $(ClC_6H_4N.CH_2)_3$	16.28	16.72	25.41	
	22.04	22.31	25.04	
	23.37	23.64	25.02	
	21.64	22.00	25.15	
	28.71	29.22	25.18	
			Av. 25.16	25.40
3-*p*-Chlorophenyl-6-chloro-3, 4-dihydroquinazoline	21.42	22.11	25.54	
	24.70	25.15	25.19	
			Av. 25.32	25.59
o-Chloroaniline samples in gelatin capsules	33.78	38.00	27.83	
	35.91	40.08	27.61	
			Av. 27.72	27.79
p-Bromoaniline	48.50	53.25	46.72	
	46.66	50.81	46.34	
	37.22	40.84	46.69	
			Av. 46.58	46.47
p-Dibromobenzene	16.73	26.70	67.91	
	22.40	35.42	67.29	
	34.40	54.64	67.59	
			Av. 67.60	67.75
Methylene-bis-*p*-bromoaniline (1) Alkaline solution filtered, $AgNO_3$ added, acidified, N_2H_4 added	12.96	14.53	47.71[c]	44.89
	21.68	23.37	45.87	
	18.68	20.25	46.13	
	14.91	16.38	46.75	
(2) As in (1), but large excess of HNO_3	24.62	26.00	44.94[d]	
	27.19	28.93	45.28	...
(3) Hydrazine added to alkaline solution; recommended procedure	12.98	13.62	44.66	
	28.96	30.60	44.93	...
			Av. 44.80	...
N-(2-benzalamino-5-bromobenzyl)-4-chloroaniline	24.60	20.81	36.00	
	28.88	24.60	36.28	
	30.54	25.71	35.83	
			Av. 36.04	35.99
3-*p*-Bromophenyl-6-bromo-3,4-dihydroquinazoline	26.47	27.08	43.54	
	26.45	27.05	43.52	
			Av. 43.53	43.66
n-Butyl iodide, b. p. 130.4–131.3° corrected. Samples in gelatin capsules	21.68	27.59	68.79	
	26.48	33.69	68.77	
	25.95	33.06	68.87	
			Av. 68.81	68.98
o-Iodobenzoic acid (1) Alkaline solution filtered and black residue washed with water	28.93	23.27	43.48[e]	51.17
	43.58	36.12	44.82	
	22.56	13.44	32.20	
(2) Alkaline solution filtered and black residue dissolved in dilute HNO_3	42.10	39.75	51.04[e]	
	20.76	19.59	51.01	
			Av. 51.03	
(3) Filtered alkaline solution treated with $AgNO_3$, then acidified and N_2H_4 added	20.87	19.72	51.08	
	52.06	49.07	50.95	
			Av. 51.02[f]	
(4) Reduction by hydrazine in alkaline solution; liquid filtered, acidified, $AgNO_3$ added. Recommended procedure	24.58	23.30	51.24	
	28.12	26.73	51.38	
	23.63	22.31	51.14	
	25.99	24.43	50.81	
			Av. 51.14	

[a] Weighings made on semimicrobalance.
[b] Blanks deducted.
[c] Results by this procedure are all much too high, owing to contamination of AgBr by precipitation in alkaline solution.
[d] Excess nitric acid decreased but did not eliminate contamination of AgBr precipitated in alkaline solution.
[e] Black residue removed by filtration of alkaline extract contained iodine, which was recovered by washing with dilute nitric acid.
[f] Iodine was satisfactorily determined by this procedure, in which only the small precipitate of AgI formed in alkaline liquid is subject to contamination, most of iodine (initially present as iodate) being precipitated later after reduction by hydrazine in acid solution.

excess sodium peroxide, an operation which is shortened if some granular Alundum (grain size 16) is introduced (*5*).

If only chlorine is to be determined acidify the cooled solution by cautious addition of 10 ml. of concentrated nitric acid, which is run in slowly along a rod extending obliquely into the covered beaker. If bromine or iodine is to be determined, introduce into the hot (not boiling) alkaline liquid 1 gram of pure hydrazine sulfate, stir to dissolve the salt, and heat the solution just below boiling until the slow effervescence ceases (about an hour for reduction of iodate and a shorter time for bromate). Cool the solution, and acidify by adding 10 ml. of concentrated nitric acid in the manner outlined above. Approach the neutral point carefully, with the liquid not more than moderately warm. If a yellow or brown color appears when the solution becomes acid the reduction of bromate or iodate was probably incomplete. In this case the prompt addition of a little hydrazine sulfate may save the analysis.

Filter the acidified solution through paper, collecting the filtrate in a 250-ml. beaker. Wash the paper with hot water, collecting the washings with the main liquid. Add water if needed to bring the volume to 125 ml. Introduce slowly and with stirring 5 ml. of 1.7 per cent silver nitrate solution. Boil the liquid for some minutes to coagulate the precipitate, and allow to stand for several hours or overnight away from strong light. The analyses reported below were allowed to stand overnight.

Decant the liquid through a 10-ml. Gooch crucible, and wash the precipitate in the beaker several times with 1 to 100 nitric acid by decantation. Transfer the precipitate completely to the filter, cleaning the beaker by use of a small policeman and a fine stream of 1 to 100 nitric acid, and wash the precipitate on the filter twice with 1 to 100 nitric acid. Using very light suction, wash filter and precipitate with alcohol and then with ether. Dry the crucible and precipitate at 130–140° for an hour, transfer to a desiccator, and when the crucible has cooled to room temperature set it in the balance case. After 10 to 15 minutes weigh the crucible to 0.02 mg. Repeat the drying, etc., until it is certain that the weight is constant within 0.03 mg.

Conduct several blank analyses in an identical manner, using no sample but 0.2 gram of sucrose. The precipitate of silver chloride may be barely visible, and it must be allowed to stand overnight before filtration, or it may be incompletely retained on the filter. In the work described the best obtainable grade of sodium peroxide was used, and the blanks for two lots were as follows: (1) 0.52, 0.46, 0.53 mg., average 0.50 mg.; (2) 0.32, 0.35, 0.40 mg., average 0.36 mg. If hydrazine sulfate is used in the analysis, a like amount must be included in the blank unless the salt is entirely free of chloride.

Results obtained by the procedure described are presented in Table I, which includes also the results of experimental trials made to test several points mentioned in the introduction.

Determination of Halogen in Liquid Substances Weighed and Decomposed in Glass Ampoules

The uncertainties introduced when the sample is contained in a glass ampoule include possible protection of the sample from complete decomposition if the ampoule is not broken before or during the mixing of the charge, and the interference by the silicic acid which may separate when the alkaline extract is acidified. The presence of silicic acid excludes any convenient procedure for the accurate gravimetric determination of halogen, and it interferes with a volumetric procedure which requires filtration to remove silver halide, for the complete washing of a precipitate which contains gelatinous silicic acid may not be feasible. Trials were therefore made using the Volhard volumetric method with the filtration omitted, the back-titrations being made in the presence of the precipitate of silver halide and silicic acid and of nitrobenzene to protect the silver halide (*4*). Blanks were determined by the same procedure. The silver nitrate and thiocyanate solutions were 0.04 *N* and 0.02 *N*, respectively.

Results by this procedure were satisfactory in the cases of *o*-chlorobenzoic acid (decomposed in presence of, but not in, a glass ampoule) and *o*-chloroaniline introduced in a glass ampoule. For *o*-chlorobenzoic acid results were 22.74 and 22.64 per cent chlorine; calculated 22.65 per cent. For *o*-chloroaniline results were 27.73, 28.08, and 27.90 per cent chlorine; calculated 27.79 per cent. Analyses of *n*-butyl chloride, chlorobenzene, and bromobenzene, all introduced in glass ampoules, gave results many of which were too low. In some cases this could be attributed to incomplete decompositions. In the determination of bromine accuracy was unavoidably decreased, owing to the analytical disadvantage inherent in the small volumes of the effective titrations. It appears from the results of these trials that a procedure which involves decomposition of the sample introduced in a glass ampoule, and determination of halogen by the Volhard titration without filtration—i. e., in presence of the precipitate of silver halide and of silicic acid—is practicable, provided complete decomposition of the sample is assured, though results for bromine and iodine may be expected to be less accurate and concordant than is desirable. The use of gelatin capsules as containers for liquid, volatile, or hygroscopic substances, and the gravimetric determination of the halogens are recommended as more satisfactory procedures.

Work is now in progress on the extension of the use of the semimicrobomb for the determination of phosphorus and of arsenic in organic compounds.

Summary

A gravimetric procedure is described for the semimicrodetermination of chlorine, bromine, and iodine in organic compounds following decomposition in the Parr sodium peroxide semimicrobomb. The volumetric semimicrodetermination of halogens by the Volhard titration is not recommended, especially for bromine and iodine, as the effective titrations, using solutions of such strengths that satisfactory end points are obtained, are too small to ensure results of acceptable accuracy and consistency.

The determination of halogen in liquid samples weighed and introduced in glass ampoules may be obstructed by the protective effect of the containers, which may be the cause of incomplete decompositions, and by the precipitation of silicic acid when the alkaline liquid from the decomposition is acidified. These disadvantages are avoided by use of small gelatin capsules for liquid samples.

Acknowledgment

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Spectrographic Limit of Identification of Potassium

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IT IS well known (*5*) that spectrographic tests are of high sensitivity for elements in the first two columns of the periodic table and of low sensitivity for elements in the last few columns of the periodic table, and (*2, 4, 7, 9*) that the intensities of the emission lines of any element are a function of the spectrographic and photographic techniques used, the matrix in which the element appears, the anions with which it is associated (*3*), and the intrinsic brightness of its lines.

There is general agreement among spectroscopists in this country that the direct current arc is the most satisfactory source to use in qualitative spectrographic analysis, and that when dealing with very small amounts of materials the lower electrode, which holds the sample, should be the cathode (*7, 9*). In the case of potassium, Steadman, Hodge and Horn, (*8*) have reported that they could detect from 1 to 5 micrograms in the direct current arc, using the potassium lines 4044.1 and 4047.2 Å. Using these lines, however, the authors found that the spectrographic sensitivity of potassium varied by factors of several hundredfold and that, in general, the sensitivity was not so great as it was reported to be. They therefore investigated the spectrographic limit of detection of potassium in the direct current arc for both the sensitive (*6*) doublets:

λ	Int.		λ	Int.
4044.14	800	and	7664.91	9000
4047.20	400		7698.98	5000

the strongest lines of the element, arising from the lowest energy values. These lines will hereafter be referred to simply as the doublets at 4000 Å. and at 7600 Å. These latter lines are seldom used because specially sensitized plates are required to photograph them, and the dispersion of prism spectrographs is low in that wave-length region.

Procedure

The spectrograms were taken in the first order of a 3-meter grating spectrograph (*1*), dispersion 5.6 Å. per mm. The 4000 Å. doublet was photographed on Eastman 33 plates, the 7600 Å. doublet on Eastman Spectroscopic I-N plates. Both types of plates were processed in accordance with the manufacturer's directions. When photographing the 4000 Å. doublet the overlapping second order spectrum was absorbed by a Corning No. 738 filter placed before the slit; in the case of the 7600 Å. lines by a Wratten Cine-Red filter. An enlarged image of the arc was formed on the slit of the spectrograph with a quartz lens and was so adjusted that the image of the cathode fell just off the edge of the Hartmann slide delimiting the slit length.

The direct current arc was operated at 15 amperes (250-volt input). The voltage across the electrodes varied from 40 to 50 volts, depending on the sample being arced. The interelectrode gap was maintained manually at 3 mm. as the electrodes burned away, with the aid of an enlarged image formed on a target by an auxiliary lens. The electrodes were 0.6-cm. (0.25-inch) diameter spectrographic graphite electrodes manufactured by the National Carbon Co. In the lower (cathode) electrode a crater 5 mm. wide and 4 mm. deep was drilled by a special fixture. The upper electrode was pointed in a pencil sharpener reserved exclusively for this purpose.

For each sample a fresh pair of electrodes was introduced in the arc stand, and preburned for 40 seconds to volatilize any traces of metal that might have been introduced in preparing the electrodes. Then a 40-second spectrogram was taken to record any impurities intrinsically present in the electrodes, and the cathode was removed and cooled rapidly in a metal block. Samples containing potassium as an impurity were weighed directly into the cathode. Potassium salts were dissolved in distilled water (which had been found potassium-free) and 0.1 ml. of solution of the appropriate concentration was placed in the cathode, which was then dried at 110° C. The loaded electrode was replaced in the arc stand, the spectrograph shutter opened, the arc started, and the spectrograph plate-holder racked down every 40 seconds until the sample was completely consumed. The exposure time used was the optimum exposure dictated for the authors' spectrograph by the twin opposing factors of maximum exposure for faint lines and minimum exposure for the background to permit maximum visibility for faint lines. In general, the bulk of the potassium was volatilized during the first exposure.

TABLE I. LIMIT OF DETECTION OF POTASSIUM

K Introduced as	Limit of Detection 4044–4047	Limit of Detection 7664–7698	Column 2 / Column 3
	Micrograms		
KCl	120	0.6	200
KOH	180	0.08	2250
K_3PO_4	95	0.08	1200
$KMnO_4$	320	0.2	1600
$K_2Cr_2O_7$	240	0.8	300
$KC_2H_5SO_3$	200	0.2	1000
Na_2CO_3	10	0.04	250
$Na_2C_2O_4$	16	0.04	400
V_2O_5	40	0.04	1000
Glass[a]	20	0.06	330

[a] Bureau of Standards borosilicate glass, standard 93

Results

The limit of detection of potassium was studied for six of its salts and for four compounds containing it as a minor impurity. The observed limits of detection are listed in Table I. The first column tabulates the form in which the potassium was introduced in the arc, the second column lists the limit of detection for the 4000 Å. doublet, the third column lists the limit of detection for the 7600 Å. doublet, and the last column gives the ratio of the two limits of detection given in the preceding columns.

The figures represent the smallest amount of potassium (introduced in the form listed in column 1) which can always be detected with certainty. One half of these amounts may or may not be detected. One quarter of these amounts will usually, but not invariably, not be detected. Whenever one line of a doublet was present, the second line invariably was present.

The striking variation in the spectrographic sensitivity of potassium demonstrated in Table I can be attributed primarily to uncontrollable changes in the conditions of excitation in the arc produced by variations in the constituents being volatilized. This phenomenon is most prominently shown by the two samples whose major constituent is sodium. While the volatile sodium is coming off in the arc, very little carbon is volatilized from the electrodes and the cyanogen bands are correspondingly faint, thus allowing faint potassium lines to be seen. In general it may be said that the limit of detection of potassium at the 4000 Å. doublet is set primarily by the intensity of the heavy cyanogen bands (*8*) rather than by the faintness of the potassium lines. This point is further demonstrated by the fact that where the theoretical ratio of intensities of the 7600 Å. doublet to the 4000 Å. doublet is roughly 10 to 1, the observed ratio of the limits of detection at 4000 Å.

to the limits of detection at 7600 Å. (column 4, Table I) varies from 200 to 2200.

Summary

The spectrographic limits of detection of potassium in the direct current arc for the doublets 4044–4047 and 7664–7698 have been studied for six potassium salts and four compounds containing potassium as minor impurities. The limits of detection vary widely, depending on the form in which the sample is introduced. The limit of detection for the 7600 Å. doublet is several hundred times lower than for the 4000 Å. band, largely because the latter are masked by heavy cyanogen bands. The limit of detection of the 4000 Å. doublet varies from 10 to 300 micrograms for the materials investigated, and for the 7600 Å. doublet it ranges from 0.04 to 0.8 microgram.

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Relation of Shape to the Passage of Grains through Sieves

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NOTWITHSTANDING the widespread use of sieves for sizing sands and other particulate materials, many problems relating to the meaning and interpretation of sieving data remain unsolved or partially solved. One such problem concerns the actual diameters of grains passed or retained by sieves. Although many factors are known to influence the size of grain passed or retained, very few data have been published, either on the net effect of these factors or on their relative importance.

Recently microprojection measurements on a natural stream sand showed that from 30 to 50 per cent of the grains retained on sieves of a $\sqrt{2}$ series had intermediate diameters larger than the rated openings of the next largest sieve (*4*). Thus the effective size of the sieve openings, as measured by the intermediate grain diameters, was considerably larger than the rated size of the openings. The passage of these oversize grains through the sieves was thought to be due mainly to two factors—(1) the relative importance of the few oversize holes in the sieves, and (2) the passage of flattened grains diagonally through the sieve openings. This article presents some data on the quantitative importance of these two factors and outlines several types of investigations in which shape-calibrated sieves can be used advantageously.

Laboratory Technique

A 60-gram sample of channel sand from the Rio Grande near Los Lunas, N. M., was placed in a nest of standard wire-mesh sieves, in which the size of openings increased from fine to coarse approximately as the $\sqrt{2}$. After 14 minutes of shaking in a Rotap mechanical shaker, the sand retained on each sieve was removed and placed in a separatory funnel partially filled with acetylene tetrabromide (specific gravity 2.92). The "heavy" minerals that sank and the "light" minerals that floated were removed to separate filter papers, washed with alcohol, and dried. After removal of magnetite, the heavy mineral separates consisted dominantly of ilmenite, pyroxene, and hornblende, and smaller amounts of garnet, apatite, mica, and other minerals. The light mineral separates were composed of quartz, feldspar, calcite, and altered grains.

Parts of the light and heavy mineral separates were split out with an Otto microsplit (*2*), mounted in a three-dimensional measuring wedge (*3*), and placed on the stage of a petrographic microscope equipped with a mechanical stage and micrometer ocular. Each grain was identified and the long diameter, a, the short diameter, c, and the intermediate diameter, b_s, that controls passage of grains through sieve openings were measured. The ratio of breadth to length, b_s/a, and the flatness ratio, c/b_s, were computed for each grain. These ratios are dimensionless numbers that have a possible range from one to zero.

All grains measured in each size grade were classified according to flatness ratio (0.999–0.900, 0.899–0.800, etc.) and the average intermediate diameter was computed for each flatness class. The averages for the smaller flatness ratios were based mainly on measurements of heavy mineral grains.

These data are presented in Table I and Figure 1. For comparison, the arithmetic mean diameter of each size grade,

Table I. Average Intermediate Diameters for Different Flatness Ratios

Flatness Ratio	Size Grade 0.351–0.246 mm. Grains measured	Average intermediate diameter	0.246–0.175 mm. Grains measured	Average intermediate diameter	0.175–0.124 mm. Grains measured	Average intermediate diameter	0.124–0.088 mm. Grains measured	Average intermediate diameter
	No.	*Mm.*	*No.*	*Mm.*	*No.*	*Mm.*	*No.*	*Mm.*
0.999–0.900	48	0.314	45	0.228	57	0.166	70	0.115
0.899 0.800	67	0.326	61	0.243	71	0.175	83	0.128
0.799–0.700	99	0.341	82	0.252	102	0.184	100	0.135
0.699–0.600	83	0.358	83	0.264	108	0.193	93	0.138
0.599–0.500	57	0.367	48	0.277	73	0.200	42	0.143
0.499–0.400	26	0.385	30	0.267	28	0.210	42	0.151
0.399–0.300	8	0.408	6	0.296	11	0.227	11	0.153
0.299–0.200	15	0.419	2	0.290	4	0.211	...	...
0.199–0.100	9	0.425	..	...	6	0.258	...	...

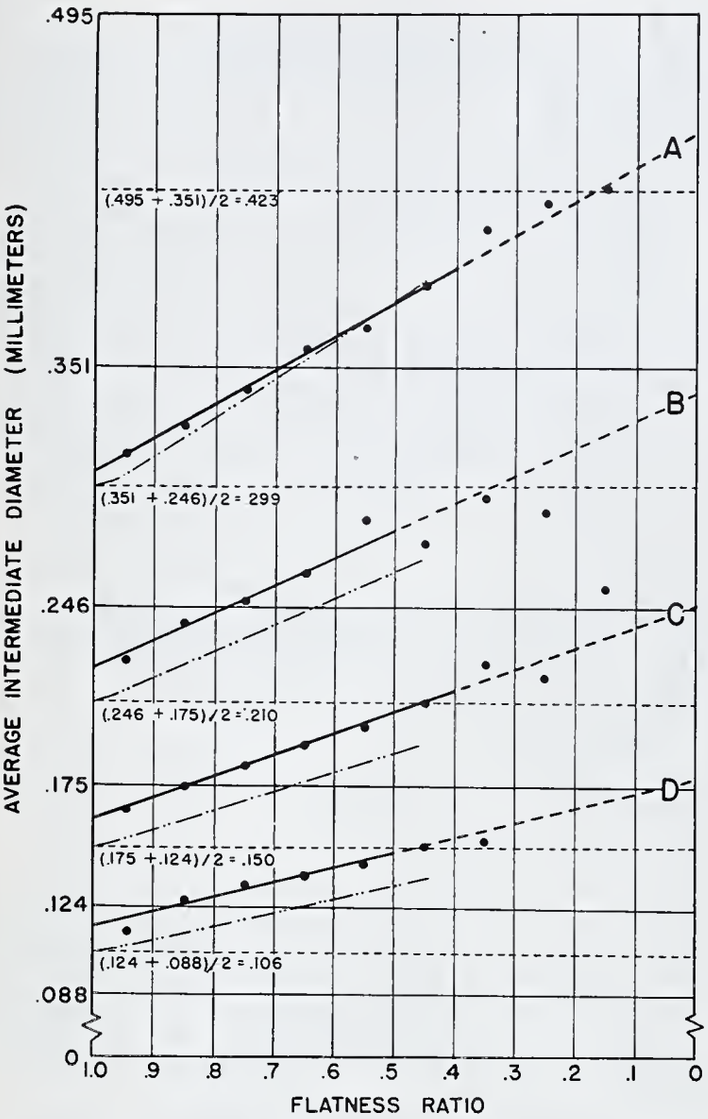

FIGURE 1. RELATION OF FLATNESS RATIO, MEASURED INTERMEDIATE DIAMETER, AND ARITHMETIC MEAN INTERMEDIATE DIAMETER

A, *B*, *C*, and *D* are for 0.351- to 0.246-mm., 0.246- to 0.175-mm., 0.175- to 0.124-mm., and 0.124- to 0.088-mm. size grades, respectively.

computed by averaging the rated openings of the two limiting sieves, is shown in Figure 1 by dashed horizontal lines.

Discussion

It is readily apparent from Figure 1 that (1) the average intermediate diameter, b_s, of particles in each sieve fraction is inversely proportional to the flatness ratios; (2) the relationship is linear when plotted arithmetically; (3) at 1.00 flatness the measured intermediate diameter is slightly larger than the arithmetic mean intermediate diameter; and (4) the flatness ratio at which the measured intermediate diameter is the same as the arithmetic mean intermediate diameter of the next larger size grade increases with decreasing grain size.

Apparently there are two ways in which shape is related to the size of grain that will pass or be retained by a particular sieve. First, if all the openings in a sieve were square and were exactly the same size, flattened grains would pass diagonally through the openings. Secondly, in the manufacture of sieves, some variation in the size and shape of openings is unavoidable. Consequently, the size and shape of grains passed or retained will be related to the number, size, and shape of the offsize openings.

The extent to which diagonal passage will occur depends, not only on the flatness ratio, but also on the cross section of the particle in the b_s–c plane. If the particles were rectangular in cross section, only those with flatness ratios less than 0.40 would be passed diagonally through holes too small to pass them in the normal way. In the case of grains of elliptical or rhombic cross section, however, grains of higher flatness ratios would be passed diagonally. The maximum effect would be for particles of rhombic cross section. For such grains, the relation between intermediate diameter and flatness has been determined graphically, and is indicated for each size grade by a dash-dot curve in Figure 1. In these determinations, the grains were assumed to lie in their most stable position on a plane surface, the same assumption made in measuring the actual particles. For particles with flatness ratios approximating zero, the limiting intermediate diameter would be $\sqrt{2}$ times the rated openings.

The difference in average intermediate diameter between the theoretical rhombic-section curves and the experimental curves of Figure 1 is the minimum that cannot be explained by diagonal passage of particles through square holes. Because most grains are not rhombic in cross section, the actual differences due to offsize openings are probably much greater, particularly in the flatness range from 0.9 to 0.6. Cross-sectional measurements were not made during the present investigation, and consequently the effect of diagonal passage of grains cannot be evaluated more precisely at this time. Whatever the actual relations between flatness ratio, intermediate diameter, and arithmetic mean intermediate diameters may be for a particular substance passing square holes, that relation would be the same for all size grades, except as the probability of grains passing the sieves is affected by the relative area of wire and mesh (*1*).

Of the offsize openings in sieves, those that are oversize will be more important than those that are undersize in determining the size and shape of grains passed or retained. Thus, if all offsize holes were square and were present in the same relative proportions in the different sieves, the measured average intermediate diameter would increase, but the new relation between flatness ratio, intermediate diameter, and arithmetic mean intermediate diameter would be the same for all size grades. But actually there will be many more offsize rectangular openings than offsize square holes, and more variation will occur in sieves of fine mesh than in sieves of coarse mesh. In consequence, oversize grains of low flatness ratio will be passed in greater numbers, and this effect will be more pronounced as the sieve mesh becomes finer. This is shown in Figure 1, where the flatness ratio at which the average intermediate diameter equals the arithmetic mean intermediate diameter of the next larger size grade increases from about 0.17 for the 0.351 to 0.246-mm. size grade to about 0.45 for the 0.124 to 0.088 mm. size grade.

The grain measurements also were analyzed to determine the relation, if any, between the breadth/length ratio and the size of grains passed or retained. No definite relation was found. Few of the grains had breadth/length ratios less than 0.50, however, and consequently the data did not provide satisfactory information regarding extremely elongated particles.

From the data presented, there seems little doubt that sets of sieves may be calibrated for both size and shape from three-dimensional measurements of particulate materials. For much routine work, the time required for calibration by methods described above will be prohibitive. Since much of the difference between measured and arithmetic mean intermediate diameters is due to the oversized openings in the sieves, it may be possible to simplify and shorten shape calibration by establishing a direct relationship between experimental curves of the type shown in Figure 1 and the standard

deviations of the offsize openings as determined by the method of Weber and Moran (*5*). Unfortunately, such a relationship could not be established by the present investigation, which was a by-product of a larger problem. The shape data were obtained several years after the sample was sized, and the sieves, though still available, had been used so much during the intervening period that any relationships based on measurements of their openings at the later data would be of questionable validity. There is also the possibility that standard samples can be used effectively in shape calibration of sieves.

For some research problems, however, shape-calibrated sieves may yield information that will amply justify the time required for calibration. One such problem is the correlation of textural data obtained by sieving, elutriation or sedimentation, and microprojection. Preliminary studies by the writer, for example, have shown that about 80 per cent of sand grains in a size grade will settle through water at the same rate as would spheres with diameters between the rated sieve openings. In the same samples, 30 to 50 per cent of the grains have intermediate diameters larger than the rated sieve openings.

Shape-calibrated sieves also can be used to provide data on the relation of grain shape to such mass properties as permeability, porosity, and strength, and to give a sounder basis for determining average grain volume, weight, or surface, and specific surface. After sieving, the long and intermediate diameters in each size grade would be measured by microprojection methods. Then, using the average intermediate diameter and the calibration curves, an average flatness ratio and an average short diameter would be obtained. Thus two-dimensional measurements, requiring no more time than other microprojection methods that are now in use, would yield three-dimensional shape data.

The curves of Figure 1 were obtained on a particular set of sieves which have certain average openings and certain dispersion of offsize openings. Sets of sieves having different average openings or different dispersion of offsize openings will require separate calibration.

Acknowledgment

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Photometric Routine Estimation of Traces of Lead by Dithizone

Application to Materials Used in the Manufacture of Cosmetics

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THE desirability of a simple method for routine analysis of raw materials for traces of lead is obvious, particularly in analytical laboratories of the food, pharmaceutical, and cosmetic industries. It is further desirable that the method involve the use of only stable, readily prepared reagents and be capable of yielding reliable results within reasonable limits of error with as few special precautions as possible.

The method of Bambach (*4*) is rapid and useful if used as recommended—i. e., as a limit test. In fact, a very similar test has been incorporated in the United States Pharmacopœia (*10*) for use on reduced iron. However, much of its advantage is lost in attempting to use it for actual routine analysis because of the necessity of daily preparation of color standards which are unstable. It would appear that recourse to one of the well-known photometric procedures (*3*, *7*, *8*) is the logical solution if a dithizone method is to be used. Hence, the method here described is an adaptation of these procedures with provision for elimination of interferences not ordinarily encountered in biological materials. The only special instrument used is a comparatively inexpensive photoelectric colorimeter, the Lumetron model 400 made by the Photovolt Corporation, which uses standard 18 × 150 mm. test tubes as sample holders. The advantage of numerous sample holders is readily apparent when several samples are run simultaneously, as is frequently the case in a control laboratory. This instrument, with one of the filters with which it is regularly equipped (transmission maximum at 530 mμ), gives very satisfactory results.

The authors have had extensive experience with the A. O. A. C. electrolytic method (*2*) and have found it very satisfactory on materials which contain at least 50 p. p. m. of lead. Materials containing less lead may, of course, be run by this method, but much time is lost in preparing the necessarily large samples. Even in the case of such substances as zinc oxide, which are most readily analyzed by the electrolytic method, the dithizone procedure here described has been found more rapid.

All the reagents used, with the exception of the dithizone solution, will keep indefinitely in glass-stoppered Pyrex bottles. If the dithizone solution is properly prepared (see reagents) and stored in the dark at 40° F., it should remain in usable condition for at least several months (*6*). Few of the reagents are specially purified, and comparatively small quan-

Table I. Acid-Extractable Lead

Substance	Sample	Lead Found	Av.	Blank	Av.	Net	Net
	Gram	*Micrograms*					*P. p. m*
TiO_2	0.5	9.5, 9.2	9.35	1.7, 1.4	1.55	7.8	16
Talc	1.0	1.6, 1.4	1.5	0.6, 0.6	0.6	0.9	0.9
Kaolin	1.0	2.3, 2.3	2.3	1.6, 1.4	1.5	0.8	0.8
$BaSO_4$	1.0	16.4, 17.4	16.9	0.7, 0.4	0.6	16.3	16

Table II. Recovery of Added Lead, Acid-Extractable

Substance	Sample	Lead Av., Table I	Lead Added	Lead Found	Av.
	Gram	*Micrograms*		*Micrograms*	
TiO_2	0.5	7.8	4	11.8, 11.9	11.85
Talc	1.0	0.9	2	2.8, 3.0	2.9
Kaolin	1.0	0.8	2	2.6, 2.9	2.75
$BaSO_4$	1.0	16.3	5	18.3, 18.4	18.35

tities of these are used, so that preparation of reagents is not frequently necessary. While more accurate results may be obtained by the use of lead-free reagents, the authors have obtained acceptable results within the desired magnitude of error with ordinary reagent grade chemicals. It is important, however, to run blanks carefully in all determinations.

The method as set forth below is specific for lead, thallium, and bismuth. Bismuth, if present, may be readily removed by extraction with dithizone at pH 2 (*11*). No sample of any of the materials described in this article has been found to contain bismuth and the authors have accordingly not felt justified in including a separation step in the procedure. Instead, it is recommended that only such samples as appear to be unusually high in lead be examined for bismuth which, if present, may then be removed as suggested. The presence of thallium, which is determined with the lead except when hydrogen sulfide is used in preparation of the sample, is extremely unlikely. Thallium may, of course, be eliminated by a preliminary hydrogen sulfide isolation of the lead. However, the authors have not yet encountered a single sample which contained thallium.

The accuracy and reproducibility of results obtained by this procedure are definitely within 2 micrograms and very probably, certainly in the case of acid-extractable lead and for acid-soluble substances, within 1 microgram.

Typical analyses, in duplicate, are shown in Tables I and III. Known amounts of lead were also added to these same samples as a check on the results (Tables II and IV). In the case of barium sulfate, poor recovery of added lead is obtained (Table II, acid-extractable), probably because of adsorption, since good recovery is obtained in the determination of total lead.

It is felt that the distinction between acid-extractable and total lead is very important and has not been given sufficient consideration in the past. The U. S. Food and Drug Administration, for example, sets a limit of 20 parts per million on lead in cosmetic materials but fails in most cases to specify how this is to be determined. It is common practice, in cases of acid-insoluble substances, to determine only acid-extractable lead. This seems justified on the assumption that lead which is not extractable by hot acid will probably produce no dermatological effects. It is, therefore, recommended that for ordinary control work only acid-extractable lead be determined, although procedures for total lead are included here.

Reagents

Water. Distilled water is redistilled in Pyrex. The use of rubber stoppers or corks is avoided.

Ammonium citrate. Ammonium citrate is prepared for use according to the directions of Bambach (*3*).

Potassium cyanide. A 10 per cent solution is prepared according to the directions of Bambach (*3*) with the following variation: The saturated, deleaded solution is boiled briefly before dilution. This serves to eliminate chloroform and prevents the solution from darkening on standing.

Hydroxylamine hydrochloride. A 20 per cent solution is prepared by the method of Bambach (*3*).

Ammonia-cyanide solution, 75 cc. of concentrated ammonium hydroxide and 100 cc. of 10 per cent lead-free potassium cyanide, made up to 500 cc. with redistilled water (*7*).

Chloroform. Chloroform is reclaimed for use by the method of Biddle (*5*). The alcohol used as preservative is freshly distilled over potassium hydroxide and is placed in the receivers before distillation of the chloroform (*6*).

Dithizone. Dithizone is purified by the conventional method (*1*). A purified product is now available (Eastman Kodak Co.) and may be used directly.

Dithizone in chloroform, 12.0 mg. per 1000 ml. by direct weight, is stored in a refrigerator.

Nitric acid, concentrated, 1 to 1, and 1 per cent (10 ml. of concentrated nitric acid plus water to make 1000 ml.). The reagent grade acid is not purified.

Sulfuric acid, concentrated and 1 to 1. The reagent grade acid is not purified.

Ammonium hydroxide, hydrochloric acid, sodium sulfate, and potassium bisulfate. Reagent grade chemicals are used directly.

Copper sulfate solution, 0.16 gram of C. P. copper sulfate per 100 cc.

Hydrofluoric acid, reagent grade.

Standard lead solution (for calibration). Recrystallized lead nitrate is dissolved in 1 per cent nitric acid and diluted to 1 or 2 micrograms per ml. with 1 per cent nitric acid.

Table III. Total Lead

Substance	Sample	Lead Found	Av.	Blank	Av.	Net	Net
	Grams	*Micrograms*					*P. p. m.*
$MgCO_3$	2.0	7.8, 8.0	7.9	2.2, 2.5	2.3	5.6	2.8
ZnO	0.1	2.9, 2.9	2.9	1.4, 1.2	1.3	1.6	16
Zinc stearate	0.5	9.2, 8.4	8.8	2.3, 2.0	2.2	6.6	13
Ocher[a]	0.1	4.2, 3.6	3.9	0.2, 0.2	0.2	3.7	37
$CaCO_3$	5.0	5.4, 5.0	5.2	5.0, 4.6	4.8	0.4	0.1
TiO_2	0.2	14.6, 14.0	14.3	3.6, 3.4	3.5	10.8	54
Talc	2.0	18, 16.3	17.15	14.8, 14.1	14.45	2.7	1.4
Kaolin	0.5	14.8, 14.0	14.4	6.0, 5.1	5.5	8.9	18
$BaSO_4$	1.0	27.0, 27.4	27.2	3.0, 2.6	2.8	24.4	24

[a] An iron oxide pigment.

Procedure for Preparation of Sample

Reasonable precautions to avoid contamination should be taken and, in any event, blanks should be treated exactly like the samples. Only Pyrex, Vycor, or platinum containers should be used. All containers should be washed with hot 1 to 1 nitric acid and thoroughly rinsed before use.

Acid-Extractable Lead. The sample is digested with nitric acid and the lead determined on the extract.

Total Lead. The entire sample is brought into solution according to the methods described below. In the case of acid-soluble substances, the results are for total lead.

Magnesium Carbonate. Dissolve 2.00 grams of the sample in 10 ml. of 1 to 1 nitric acid. Boil to eliminate carbon dioxide, cool, and dilute to about 75 cc. in a separatory funnel. Add 3 ml. of ammonium citrate solution and make alkaline to phenol red with ammonium hydroxide. Add 0.5 ml. of hydroxylamine hydrochloride solution and 1 ml. of potassium cyanide solution.

Zinc Oxide. Dissolve 0.100 gram of the sample in 1 ml. of 1 to 1 nitric acid and wash the solution into a small separatory funnel with several portions of water. Add 3 ml. of ammonium citrate solution and make alkaline to phenol red with ammonium hydroxide. Add 0.5 ml. of hydroxylamine hydrochloride solution and 10 ml. of potassium cyanide solution.

Zinc Stearate. Ash 0.500 gram of the material in a silica or Vycor crucible in a muffle furnace at 475° to 500° C. for 15 to 20 minutes. Cool and wet the residue with 3 drops of concentrated nitric acid. Evaporate to dryness over a low flame and return to the muffle for 20 to 30 minutes. A clean white ash should result. The nitric acid treatment may be repeated if

necessary. Dissolve in 1 ml. of 1 to 1 nitric acid and wash into a small separatory funnel with several portions of water. Add 3 ml. of ammonium citrate solution and 0.5 ml. of hydroxylamine hydrochloride solution and make alkaline to phenol red with ammonium hydroxide. Then add 10 ml. of potassium cyanide solution.

Cosmetic Ochers (ferric oxides). Boil 0.100 gram with 2 ml. of concentrated hydrochloride until dissolved. Add 3 drops of nitric acid and boil to eliminate chlorine and oxides of nitrogen, adding a little water if necessary. Do not allow the solution to go to dryness. Transfer to a separatory funnel and add 5 ml. of ammonium citrate solution and 5 ml. of hydroxylamine hydrochloride solution. Make alkaline to phenol red with ammonium hydroxide and add a 2-ml. excess. Cool thoroughly and add 6 ml. of potassium cyanide solution.

Calcium Carbonate. Dissolve 5.00 grams of the sample in 15 ml. of 1 to 1 nitric acid, boil, and cool the solution. Add 5 ml. of ammonium citrate solution and 5 ml. of copper sulfate solution and adjust the pH to 3.0 to 3.4 (bromophenol blue) with ammonium hydroxide. Saturate with hydrogen sulfide, filter, and wash with 3 portions of 3 per cent sodium sulfate solution. (The pH of a 3 per cent solution of sodium sulfate solution is adjusted to 3.0 to 3.4 and the resulting liquid saturated with hydrogen sulfide before using it as a wash.) Dissolve with 4 ml. of hot 1 to 1 nitric acid, catching the filtrate in the same flask in which the precipitation was carried out. Wash with six small portions of hot water, adding the washings to the filtrate. Boil to eliminate sulfide and transfer to a small separatory funnel. Add 3 ml. of ammonium citrate solution and make alkaline to phenol red, adding a 0.5-ml. excess. Add 0.5 ml. of hydroxylamine hydrochloride solution and 1 ml. of potassium cyanide solution. [It is possible to hold calcium in solution, at least temporarily, at this pH in the presence of ammonium citrate. However, in the case of calcium carbonate, where a rather large (5.0-gram) sample is advisable because of low lead content, a sulfide separation is preferable to the use of the very large amounts of ammonium citrate and large volumes of solution which otherwise become necessary.]

Table IV. Recovery of Added Lead, Total Lead

Substance	Sample	Lead Av., Table III	Lead Added	Lead Found		Av.
	Grams	*Micrograms*				
$MgCO_3$	2.0	5.6	5	10.9,	9.1	10.0
ZnO	0.1	1.6	2	3.5,	3.9	3.7
Zinc stearate	0.5	6.6	5	11.4,	10.5	11.0
Ocher	0.1	3.7	5	8.8,	8.8	8.8
$CaCO_3$	5.0	0.4	5	5.7,	5.3	5.5
TiO_2	0.2	10.8	4	13.9,	13.3	13.6
Talc	1.0	1.4[a]	5	6.6,	6.2	6.4
Kaolin	0.5	8.9	4	14.1,	11.9	13.0
$BaSO_4$	0.5	12.2[a]	5	16.0,	17.1	16.6

[a] Calculated.

Titanium Dioxide. Acid-Extractable Lead. Add 0.500 gram to 10 ml. of 1 to 1 nitric acid in a 15-ml. graduated centrifuge tube, digest 1 hour in boiling water bath with frequent agitation, centrifuge, and pour off the liquid into a small separatory funnel. Add 5 ml. of 1 to 1 nitric acid and 5 ml. of water to the centrifuge tube, break up the packed mass with a glass rod, and digest for 15 minutes on the water bath. Centrifuge and add the liquid to the original extract. Add 3 ml. of ammonium citrate solution and make alkaline to phenol red with ammonium hydroxide, adding a 0.5-ml. excess. Add 0.5 ml. of hydroxylamine hydrochloride solution and 1 ml. of potassium cyanide solution.

Total Lead. Fuse 0.200 gram of the titanium dioxide with 2.00 grams of potassium bisulfate in a covered Vycor crucible. Use only a Bunsen flame and heat only until a clear melt is obtained. Rotate the crucible as the melt solidifies, so as to spread it in a thin layer over the sides. After the melt has cooled, add 4 ml. of ammonium citrate solution and 5 to 6 ml. of water. Heat carefully until a clear solution is obtained. Transfer to an Erlenmeyer flask, washing well with a few portions of water, cool, and adjust the pH to 3.0 to 3.4 (bromophenol blue) using dilute sulfuric acid. Add 5 ml. of copper sulfate solution.

Saturate with hydrogen sulfide, filter, and wash thoroughly (ten times) with 3 per cent sodium sulfate solution. (The pH of a 3 per cent solution of sodium sulfate solution is adjusted to 3.0 to 3.4 and the resulting liquid saturated with hydrogen sulfide before using it as a wash.) Dissolve the precipitate with 4 ml. of hot 1 to 1 nitric acid, catching the filtrate in the flask in which the precipitation was effected. Wash the paper with six small portions of hot water, adding the washings to the filtrate. Boil to eliminate sulfide and transfer to a small separatory funnel. Add 3 ml. of ammonium citrate solution, and make alkaline to phenol red with ammonium hydroxide, adding a 0.5-ml. excess. Add 0.5 ml. of hydroxylamine hydrochloride solution and 1 ml. of potassium cyanide solution. [Titanium is not precipitated at the pH of extraction in the presence of citrate, but even small amounts completely prevent the extraction of lead by dithizone (see Table V[1]). Hence a preliminary isolation of the lead as sulfide was resorted to. The precipitate of lead sulfide and copper sulfide must be washed very thoroughly to remove all trace of Ti^{++++}. Since the amount of Ti^{++++} necessary to interfere with the lead extraction is small, it was thought that double precipitation of the sulfides would be advisable. However, experiments showed this to be unnecessary, providing the sulfides are well washed.]

Talc. Acid-Extractable Lead. Follow the procedure for titanium dioxide exactly, using a 1.00-gram sample.

Total Lead. To a platinum crucible containing 3 grams of hydrofluoric acid and 6 ml. of 1 to 1 sulfuric acid, add 1.00 gram of the talc. Place in a sand bath heated to 200° to 250° C. and take to fumes of sulfur trioxide. Cool, add 1 gram of hydrofluoric acid, and again heat to sulfur trioxide fumes. Repeat once more with 0.5 gram of hydrofluoric acid. [The sample of talc must be fumed strongly for some minutes to drive off excess fluoride. If much fluoride ion remains it is difficult to obtain a clear solution.] Cool, dilute with a little water, wash into a separatory funnel, add 3 ml. of ammonium citrate solution, and make alkaline to phenol red with ammonium hydroxide, adding 0.5-ml. excess. Add 0.5 ml. of hydroxylamine hydrochloride solution and 1 ml. of potassium cyanide solution.

Kaolin. Acid-Extractable Lead. Follow the procedure for titanium dioxide but use 5 per cent nitric acid for both extractions in place of the more concentrated acid and use a 1.00-gram sample. [When the acid concentration is too high (1 to 1), considerable aluminum is extracted. This leads to poor results.]

Total Lead. To a platinum crucible containing 1.5 grams of hydrofluoric acid and 5 ml. of 1 to 1 sulfuric acid, add 0.500 gram of the kaolin. Place in a sand bath at 200° to 250° C. and heat to fumes of sulfur trioxide. Cool, add 5 drops of nitric acid to destroy organic matter, and again heat to sulfur trioxide fumes. Transfer to an Erlenmeyer flask with about 25 ml. of water, add 5 ml. of ammonium citrate solution, and boil for a few minutes until a clear solution is obtained. Cool and adjust the pH to 3.0 to 3.4 (bromophenol blue). Add 5 ml. of copper sulfate solution.

Saturate with hydrogen sulfide, filter, and wash 5 or 6 times with 3 per cent sodium sulfate solution. (The pH of a 3 per cent sodium sulfate solution is adjusted to 3.0 to 3.4 and the resulting liquid saturated with hydrogen sulfide before using it as a wash.) Dissolve with 5 ml. of boiling 1 to 1 nitric acid, catching the filtrate in the flask in which the precipitation was effected. Wash with six portions of hot water, adding the wash to the filtrate. Boil to eliminate hydrogen sulfide, cool, and transfer to a small separatory funnel. Add 3 ml. of ammonium citrate solution and make alkaline to phenol red with ammonium hydroxide, adding a 0.5-ml. excess. Add 0.5 ml. of hydroxylamine hydrochloride solution and 1 ml. of potassium cyanide solution. [The extraction of lead in the presence of much aluminum is very unsatisfactory (see Table VI). A preliminary isolation of the lead as sulfide eliminates this difficulty completely.]

Barium Sulfate. Acid-Extractable Lead. Follow the procedure for titanium dioxide exactly. Use a 1.00-gram sample.

Total Lead. Mix 1.00 gram in a platinum crucible with 2 grams of sodium carbonate and 3 grams of potassium carbonate. Fuse over a Bunsen burner to a clear, mobile liquid. Cool, add a small amount of water, and heat cautiously over a flame. This will loosen the melt and enable its transfer to a beaker. Boil with 20 to 25 ml. of water until the mass is thoroughly disintegrated, filter the hot solution, and wash 6 to 8 times with 4-ml.

[1] The results shown in Tables I to V include a reagent blank of approximately 1.4 micrograms.

Table V. Effect of Titanium on Extraction of Lead by Dithizone

Lead Added	Titanium	Lead Found
Micrograms	*Micrograms*	*Micrograms*
10	0	11.4
10	10	11.4
10	500	11.4
10	5,000	11.1
10	50,000	3.4
10	100,000	0.2

portions of hot 5 per cent sodium carbonate solution until the wash liquid is free of sulfate. Finally wash twice with small portions of water, discarding this wash. To the combined filtrate and washings add 5 ml. of ammonium citrate solution, 0.5 ml. of hydroxylamine hydrochloride solution, and 1.0 ml. of potassium cyanide solution (Solution 1).

Dissolve the residue on the filter in 2 ml. of hydrochloric acid diluted with about 5 ml. of water. Wash with several small portions of water, adding the washings to the acid solution. Boil for a few minutes to eliminate carbon dioxide and transfer to a separatory funnel. Add 3 ml. of ammonium citrate solution and make alkaline to phenol red with ammonium hydroxide, adding 0.5-ml. excess. Add 0.5 ml. of hydroxylamine hydrochloride solution and 1 ml. of potassium cyanide solution. The solution at this point should have a volume of about 75 ml. and be perfectly clear (Solution 2).

Table VI. Effect of Aluminum on Extraction of Lead by Dithizone

Lead Alone		In Presence of 1.5 Grams of $Al_2(SO_4)_3.18H_2O$	
Extract No.[a]	Lead	Extract No.[a]	Lead
	Micrograms		*Micrograms*
1	10.6	1	5.3
2	1.0	2	3.8
3	0.5	3	2.0
4	0.0	4	0.7
		5	1.1
		6	0.6

[a] Successive 5-ml. portions of dithizone.

Extraction of Lead

The prepared sample is extracted with three successive 5-ml. portions of dithizone which are combined in a small (60-ml.) separatory funnel. In the case of barium sulfate (total lead), Solutions 1 and 2 are extracted separately and all extracts are combined in a single separatory funnel.

The combined extracts are then shaken with 20 ml. of 1 per cent nitric acid and the dithizone solution is discarded.

Determination of Lead

Four milliliters of the ammonia-cyanide solution and 2 drops of the hydroxylamine hydrochloride solution are added to the 20 ml. of acid containing the lead, 10.0 ml. of the dithizone are added from a buret, and the mixture is shaken for 30 seconds. The chloroform layer is then filtered through a 7-cm. acid-washed filter paper folded in eights and fitted directly into the mouth of one of a series of matched 18 × 150 mm. test tubes.

A "zero" standard is prepared by treating 10.0 ml. of the dithizone with 20 ml. of 1 per cent nitric acid containing 4 ml. of the ammonia-cyanide solution and 2 drops of hydroxylamine hydrochloride solution. This is used to set the photometer to 100 per cent transmission.

A reading is then taken on the prepared sample and on a blank which is prepared in exactly the same way as the sample, omitting only the material undergoing analysis, using the yellow-green filter (transmission maximum 5300 Å.). From these readings, the amount of lead is read from a calibration curve constructed by analysis of varying amounts of the standard lead solution.

Discussion

This procedure is not intended to replace the more accurate techniques employing dithizone (*3, 7, 8, 11*), but rather to provide a comparatively simple method for use by the average control laboratory where a precision of 1 or 2 micrograms is adequate. In such a laboratory, speed and simplicity are important factors and the use of only simple, rugged instruments is desirable.

It is felt that too little consideration is given to the possible interference of various cations in the extraction of lead by dithizone, although at least one article in the literature (*9*) deals with this question. The authors have found (see Table V and VI) that the presence of aluminum definitely interferes with the extraction of lead and the presence of titanium prevents it completely.

It has been observed in this laboratory that the presence of only 5 to 10 mg. of titanium seriously interferes with the extraction of lead by dithizone and that 0.1 to 0.2 gram prevents it completely. Thus, in analyzing a sample containing 0.2 gram of titanium dioxide, if it is attempted to extract the lead directly (without recourse to preliminary separation by hydrogen sulfide), the blank will show more lead than the sample. This effect is not due to a simple shift in the pH of optimum extraction, since experiments have shown the same results at various pH from 7.0 to 11.0.

The procedure of adding known amounts of lead to samples for analysis was preferred to spectrographic analysis as a means of checking results. Three well-known spectrographic laboratories, working with a master sample of titanium dioxide, obtained values of 24, 45, and 66 p. p. m., respectively. The authors obtained 54 p. p. m. (see Table III), using the above dithizone procedure.

As an additional check, samples of zinc oxide, talc, and titanium dioxide were analyzed by the A. O. A. C. electrolytic method (*2*). Good agreement with the authors' dithizone procedure was obtained in each case.

Summary

A simple photometric procedure for the routine determination of lead by dithizone is described. The procedure is fairly rapid, requires very little special equipment, and is sufficiently accurate for most control work (within 1 or 2 micrograms).

The method has been in use for some time on a number of common materials used in the cosmetic industry. The results have been very satisfactory and the procedure could be readily adapted to virtually any material. Detailed methods for the preparation of samples of such materials as are commonly used in the manufacture of cosmetics have been worked out. These methods of preparation could be followed by analysis according to any of the known dithizone procedures.

Aluminum and titanium have been found to interfere with the extraction of lead by dithizone.

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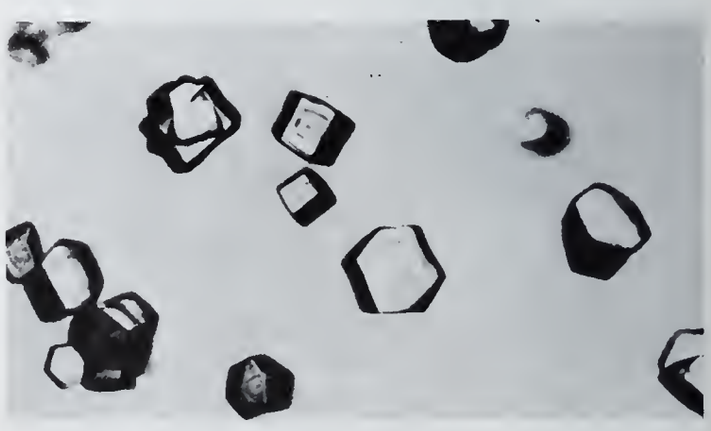

Bismuth Cobalticyanide *Courtesy, A. A. Benedetti-Pichler*

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Determination of *o*-Cresol in Phenol by a Cloud Point Method

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IN THE process of freeing phenol from *o*-cresol by distillation it is important to have an accurate method for determining low concentrations of the latter in the presence of the former, in order to evaluate the plate efficiency of the column. It is assumed from a knowledge of the compounds involved that only *o*-cresol is present with the phenol.

One method for *o*-cresol in phenol (*6*, *10*) is based upon the formation of a complex between *o*-cresol and cineole, followed by the determination of the freezing point of this complex. This seems to be more suitable for high concentrations of cresol. Another method which has been recommended by a number of authors (*3*, *7*, *8*, *11*) is based upon the lowering of the freezing point of phenol. Unfortunately, *o*-cresol freezes out with the phenol in a continuous series of mixed crystals (*3*). This would make it impossible to get very accurate values of *o*-cresol unless the degree of supercooling were rigidly controlled in relation to the concentration of *o*-cresol. The extent of this difficulty is obvious from an inspection of the divergent values reported for the lowering of the freezing point of phenol by *o*-cresol (*3*, *7*, *8*, *11*). At least one of these authors (*3*) recognized this difficulty, and so determined the temperature at which the last crystals melted, instead of the usual freezing point, in order to avoid the effects of supercooling. This would, however, make the procedure less adapted to routine use. Besides, the lowering of the freezing point for a given *o*-cresol content is considerably less than the corresponding lowering of the cloud point in the method reported below.

The statement (*1*) that 1 volume of cold liquefied phenol (rendered liquid by the addition of 8 per cent of water) forms, with 1 volume of glycerol, a clear liquid which is not rendered turbid by the addition of 3 volumes of water (absence of creosote and of cresol) suggested the possibility that a cloud point method could be developed for *o*-cresol in phenol. Dolique (*4*) has published a study of the systems phenol-water and phenol-glycerol-water, including the effects of a number of impurities. Other papers have also been published on the influence of impurities on the critical solution temperature of phenol-water (*2*, *5*, *9*), but in none did the authors find that *o*-cresol had been included.

The authors' first work was done with the system phenol-water-glycerol with additions of *o*-cresol, but this was abandoned in favor of the phenol-water system because the former involved some difficulties in the precise observation of the cloud point, and had no advantage over the simpler system. Working with mixtures of phenol and *o*-cresol containing up to 5.6 per cent cresol, and also containing water added in the ratio of 65 parts by weight to 35 parts by weight of the mixed phenol-cresol, the authors found that each addition of cresol increases the cloud point to an extent that can be expressed by two linear equations, one for concentrations from 0 to 3 per cent *o*-cresol and the other for concentrations from 3 to 5.6 per cent. The method has an accuracy for the lower concentration range which is expressed by a standard deviation of a single value from the true *o*-cresol content of ±0.07 per cent *o*-cresol and for the higher concentrations of ±0.09 per cent. The accuracy can obviously be increased by taking the average of more than one determination.

Method of Analysis

About 7 to 8 grams of the phenol sample are placed in a dry, tared test tube (2.5 × 15 cm.) and weighed to the nearest milligram. A number of milliliters of distilled water equal to 1.857 times the weight of the phenol sample (this makes a mixture of 35 per cent phenol sample and 65 per cent water) are added from a buret. The tube is placed in a water bath at 75° to 80° C. and its contents are mixed with a glass stirrer (looped to fit around the thermometer to be used later) until the mixture is clear. The tube is then centered by means of a cork in a larger test tube (3.25 × 17.5 cm., 1.5 × 7 inches) which serves as an air jacket and is kept in a water bath maintained at about 65° C. A thermometer with 0.1° divisions, calibrated for 7.5-cm. (3-inch) immersion, is placed in the solution, which is then stirred vigorously (about 80 to 100 strokes per minute).

As the solution cools, a turbidity sets in which serves as a warning of the approach of the cloud point. This turbidity starts at about 1° to 2° before the cloud point, starting sooner the greater the concentration of cresol, but its onset and the increase in its opacity are not sharp. Finally there is an abrupt increase in the opacity of the mixture. This is taken as the cloud point and the thermometer is read to the nearest 0.05° C. The tube may be reheated and the determination repeated at will if it is desired to increase the accuracy by taking a mean of several values.

For cloud points up to 70.25° C., the percentage of *o*-cresol is calculated by the equation

$$\%\ o\text{-cresol} = \frac{\text{cloud point (° C.)} - 66.40}{1.326} \qquad (1)$$

and for cloud points from 70.25° to 73.5° C. by the equation

$$\%\ o\text{-cresol} = \frac{\text{cloud point (° C.)} - 66.81}{1.167} \qquad (2)$$

Development of the Method

PHENOL was purified by distilling C. P. phenol through a packed column, redistilling the middle cut, redistilling the middle cut from this, and collecting three successive cuts which had freezing points of 40.94°, 40.93°, and 40.93° C., respectively. The freezing points were run in a magnetically stirred enclosed apparatus to protect the phenol from absorption of moisture from the air. There was no significant change in the freezing point when the phenol was boiled in the freezing point tube in order to remove possible traces of moisture.

TABLE I. DEVIATION OF CALCULATED CRESOL VALUES FROM TRUE VALUES

Cloud Point (Mean of 10 Values) ° C.	Cresol Calculated %	Cresol Actual %	Deviation % cresol
	By Equation 1		
66.39	−0.01	0.00	−0.01
66.41 (mean of 5 values)	0.01	0.00	+0.01
67.00	0.45	0.45	0.00
67.36 (mean of 5 values)	0.72	0.67	+0.05
67.70	0.98	1.03	−0.05
68.19	1.35	1.37	−0.02
68.33	1.46	1.46	0.00
69.18	2.10	2.08	+0.02
69.40	2.26	2.33	−0.07
70.23	2.89	2.83	+0.06
	By Equation 2		
70.77	3.39	3.43	−0.04
71.59	4.10	4.17	−0.07
72.34	4.74	4.71	+0.03
73.42	5.66	5.59	+0.07

o-CRESOL was prepared by diazotizing and decomposing with water *o*-toluidine which had in turn been prepared from *o*-acetotoluide that had been recrystallized to a constant melting point of 110.9–111.6° C. (corrected). The *o*-cresol was distilled under reduced pressure. The middle cut had a freezing point (run in the aforementioned closed apparatus) of 30.99° C. (corrected). The same freezing point was obtained upon redistilling this material under reduced pressure and taking a middle cut.

THERMOMETERS. The thermometers were calibrated at an immersion depth of 7.5 cm. (3 inches) by comparing with another thermometer certified by the National Bureau of Standards.

GLYCEROL-WATER-PHENOL-CRESOL MIXTURES. Choosing proportions of phenol, glycerol, and water on the basis of data reported by Dolique (*4*), a number of cloud points were determined for phenol-cresol mixtures containing up to 5.8 per cent *o*-cresol. The phenol-cresol samples were mixed with an aqueous glycerol solution having a specific gravity at $\frac{30° \text{ C.}}{30° \text{ C.}}$ of 1.0218, in the ratio of 87.80 ml. of the glycerol solution to 13 grams of the phenol-cresol mixture. It was possible to get cloud point values for the individual mixtures which agreed within 0.05° C., but when the cloud points were plotted against the cresol content, some of the points fell away from a straight line by as much as 0.2 per cent cresol. The difficulty was with the determination of the cloud point. This did not appear sharply enough, so that there was a lack of concordance between the point taken as the cloud point for one sample and that for another, although two successive readings on the same sample were concordant.

PHENOL-WATER-CRESOL MIXTURES. The first mixture which was tried consisted of 32.15 per cent water and 67.85 per cent phenol by weight. Repeated determinations of the cloud point on the same mixture resulted in successive values, each of which was lower than the previous value by about 0.2° C., the initial value being 33.75° C. According to Dolique's data (*4*) a change in the water content of the mixture of about 0.07 per cent (or for the size of sample used, about 0.004 ml. of water) would suffice to lower the cloud point by 0.2° C. It would seem most likely that such losses explained the difficulty with this mixture.

It was next decided to use a mixture of 35 per cent phenol and 65 per cent water which would be closer to the critical solution temperature of the system, because the system is much less sensitive to small changes in composition in that region.

The method proposed does not pretend to determine the critical solution temperature, which is the temperature at which the two liquid phases become equal in composition. What is determined is merely the temperature at which a definite clouding phenomenon appears. Because of this fact it is unnecessary for the use of the method to resolve the divergencies in the critical solution temperatures which have been reported in the literature.

A number of mixtures were made up of phenol and *o*-cresol, ranging from 0 to 5.6 per cent *o*-cresol, and the cloud points were determined. With each mixture ten separate determinations of the cloud point were made (five in two cases). When the average cloud points were plotted against the known cresol content, it was found that the relationships could be expressed most conveniently by two straight lines (Equations 1 and 2). Possibly the fundamental relationship could be expressed by a hyperbolic equation, but the use of two straight-line equations seems more practical. The equations were chosen in such a way that the percentages of *o*-cresol, calculated for each of the average cloud points from the equations, would, when compared with the actual percentages of *o*-cresol, give deviations such that the difference between the sum of the squares of the positive deviations and the sum of the squares of the negative deviations would be at a minimum. The data are given in Table I.

Precision and Accuracy

The precision of the method will be determined by the degree of agreement between individual cloud point values on a single mixture and the mean. This has been determined for each of the 14 mixtures by calculating for each mixture the average deviation of each value from its respective mean and then multiplying by a factor (1.49 for 5 values and 1.36 for 10 values) to convert to the standard deviation. (The calculation of the standard deviation by means of a factor is not so accurate as by means of the usual root mean square average of the deviations from the arithmetic mean, but it is felt that the difference in the two methods of calculation is not sufficient to affect the reported standard deviations in the one significant figure to which they have been calculated.) The latter is then calculated to per cent *o*-cresol, using Equation 1 or 2. The data are given in Table II.

The accuracy of a single value was determined by calculating by means of Equations 1 or 2 the cloud point value corresponding to each actual percentage of *o*-cresol, getting the average deviations of the individual determined cloud point

TABLE II. PRECISION OF CLOUD POINT DETERMINATION

Composition of Sample % *o-cresol*	Cloud Point (Mean of 10 Values) ° C.	Average Deviation from Mean ±° C.	Standard Deviation from Mean ±° C.	Standard Deviation from Mean ±% *o-cresol*
0.00	66.39	0.050	0.07	0.05
0.00	66.41 (mean of 5 values)	0.068	0.10	0.08
0.45	67.00	0.045	0.06	0.05
0.67	67.36 (mean of 5 values)	0.040	0.06	0.05
1.03	67.70	0.045	0.06	0.05
1.37	68.19	0.040	0.05	0.04
1.46	68.33	0.045	0.06	0.05
2.08	69.18	0.067	0.09	0.07
2.33	69.40	0.061	0.08	0.06
2.83	70.23	0.067	0.09	0.07
		Av.	0.07	0.05
3.43	70.77	0.052	0.07	0.06
4.17	71.59	0.050	0.07	0.06
4.71	72.34	0.046	0.06	0.05
5.59	73.42	0.065	0.09	0.08
		Av.	0.07	0.06

TABLE III. ACCURACY OF METHOD

Composition of Sample % *o-cresol*	Calculated Cloud Point ° C.	Deviation from Calculated Cloud Point: Average ±° C.	Average ±% *o-cresol*	Standard ±% *o-cresol*
0.00	66.40	0.070	0.053	0.07
0.00	66.40	0.055	0.041	0.06
0.45	67.00	0.045	0.034	0.05
0.67	67.29	0.066	0.050	0.07
1.03	67.77	0.065	0.049	0.07
1.37	68.22	0.046	0.035	0.05
1.46	68.34	0.045	0.034	0.05
2.08	69.16	0.068	0.051	0.07
2.33	69.49	0.107	0.081	0.11
2.83	70.15	0.080	0.060	0.08
			Av.	0.07
3.43	70.81	0.062	0.053	0.07
4.17	71.68	0.090	0.077	0.10
4.71	72.31	0.056	0.048	0.07
5.59	73.33	0.091	0.078	0.11
			Av.	0.09

values from their respective calculated values, recalculating the average deviation to per cent cresol, and converting to the standard deviation as per cent cresol. These data are given in Table III.

The values indicate that the precision is a little better than the accuracy. By taking the mean of a number of cloud point determinations the standard deviation may be improved by a factor equal to the reciprocal of the square root of the number of determinations.

Effect of Variations in Water Content

Since the sample must be made up by adding water, it is important to know how changes in the water content of the prepared sample will affect the cloud points. This is particularly necessary because of the hygroscopic nature of phenol. If the accuracy of the method were affected by small changes in water content, it would be difficult to use.

Dolique (*4*) published data on the change of the critical solution temperature of phenol-water with changes in composition:

Water %	Phenol %	Critical Solution Temperature ° C.
61.16	38.84	66.4
64.13	35.87	66.5
65.00	35.00	66.5
65.69	34.31	66.5
68.46	31.54	66.4

It is seen that near the region of concentrations with which this method is concerned, the cloud point of pure phenol is insensitive to small changes in water concentration.

To two mixtures of cresol in phenol, 1 per cent less and 1 per cent more, respectively, than the usual 65 per cent of water were added, with results as follows:

o-Cresol Content %	Water Content %	Cloud Point ° C.	*o*-Cresol Found %	Error % *o-cresol*
1.14	64	67.78	1.05	−0.09
	65	67.91 (calcd.)	..	....
	66	67.88	1.13	−0.01
2.63	64	69.61	2.46	−0.17
	65	69.88 (calcd.)	..	....
	66	69.84	2.62	−0.01

It can be seen that 1 per cent additional water causes a negligible error. If about 15 ml. of water are measured from a buret for a single mixture, an excess of even 0.2 ml. will cause an error of no more than 0.01 per cent *o*-cresol. A deficiency of water may be more serious, but that can be avoided with considerable certainty.

Summary

A method has been devised for the determination of *o*-cresol in phenol in concentrations up to 5.6 per cent, by measuring the cloud point of a mixture of the sample with water. The relationship between the cloud point and the *o*-cresol content is expressed by one straight line equation up to 3 per cent and another from 3 to 5.6 per cent. The accuracy of the method is indicated by the standard deviation of a single value from the true *o*-cresol content; this equals ±0.07 per cent *o*-cresol up to 3 per cent and ±0.09 per cent from 3 to 5.6 per cent.

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A New Gas Generator

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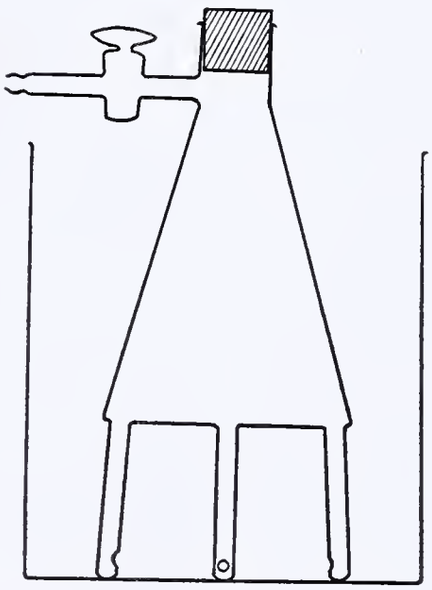
THE work has frequently required a compact, portable form of gas generator. The instrument described here was finally adopted as being satisfactory from the standpoints of simplicity of manufacture and operation and ease of clean[illegible]



For ordinary operations, a 300-ml. Erlenmeyer flask was employed, to the base of which were sealed three 5-cm. lengths of 8- to 9-mm. tubing, as illustrated. These legs were sealed off and a small hole was blown into each of them near the base. A stopcock side arm was sealed to the neck of the flask, as shown, though in a simpler modification the stopcock was simply inserted in the stopper. The instrument fits nicely into a 1-liter beaker.

In operation, the solid reagent is placed in the flask, while the acid is poured into the beaker, to within an inch of the brim. Opening the stopcock admits acid through the legs of the flask, generating the gas. When the stopcock is closed, the gas pressure displaces the acid from the flask and the reaction ceases.

Determination of Furfural

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IN STUDYING methods and procedures for the preparation of hemicellulose from plant material many obstacles are encountered. One difficulty is the possible loss of hemicellulose as the steps in the preparation are carried out. A means of determining any change in the hemicellulose content would aid considerably, as the procedure could be modified to prevent this loss. With certain types of material, furfural may be determined at the various stages of extraction and purification to indicate whether there is a change in the hemicellulose content. With this in mind methods for determining furfural were examined, seeking an accurate and rapid method which is easily manipulated.

The method of Tollens and Kröber for the determination of furfural (*6*) is the one most commonly used. The pentoses, pentosans, etc., are converted into furfural by distillation with 12 per cent hydrochloric acid solution according to a standard procedure. After precipitation with phloroglucinol the weight of furfural, pentose, or pentosan corresponding to a given weight of phloroglucide may be obtained by reference to Kröber's tables.

Pentoses do not usually yield the theoretical quantity of furfural upon distillation with acids. Xylose gives about 90 per cent and arabinose about 75 per cent of theoretical. This has been explained as being due to a partial destruction of furfural. Pervier and Gortner (*3*) and Youngburg (*7*) suggested distilling in a current of steam to lessen the opportunity for decomposition. Others (Iddles and Robbins, *2*) found that passing steam into the solution during distillation gave little or no increase in furfural.

Instead of distilling pentoses or pentosans in the presence of hydrochloric acid, Youngburg (*7*) used phosphoric acid. The distillation was carried out in a stream of steam and the temperature in the flask was allowed to rise to 175° C. Good results were claimed when pure xylose or arabinose were distilled, but only approximations are obtained in the case of various complex materials yielding furfural.

Pervier and Gortner (*3*) found that furfural can be determined in acid solution by oxidation with bromate salts in the presence of bromides, determining the end point electrometrically. They also found that the Tollens-Kröber precipitation method was in considerable error when applied to dilute solutions of furfural. Powell and Whittaker (*4*) improved the method of determining the end point by using an excess of bromate, adding potassium iodide, and titrating back with thiosulfate. Each milliliter of 0.1 *N* bromate used is equivalent to 0.0024 gram of furfural. Hughes and Acree (*1*) carried out the reaction at 0° C. This procedure held the absorption to 1 mole of bromine per mole of furfural and thus eliminated the error involved in the slow absorption of the second mole of bromine. Each milliliter of 0.1 *N* bromate used is equivalent to 0.0048 gram of furfural.

For the colorimetric estimation of furfural in the distillate such color reagents as benzidine, xylidine salts, and aniline salts have been used. Stillings and Browning (*5*) developed a method in which the furfural is allowed to react with aniline acetate in acetic acid solution and the intensity of color produced is measured photocolorimetrically.

Experimental

Since it was desired to select a suitable method for determining furfural in plant material, some of the available methods were compared. As the Tollens and Kröber method has been shown to be in considerable error, particularly when applied to low concentrations of furfural, attention was turned to a volumetric or colorimetric procedure.

A study of the colorimetric method of Stillings and Browning (*5*) showed that fairly consistent results are obtained when the temperature, concentration of reagents, and freedom from light are carefully controlled. However, the method could be improved by reducing the time required for maximum color to develop and by stabilizing the color. With this in mind a number of substances were added to the furfural solutions prior to the addition of the color reagent. It was found that small amounts of a mixture of oxalic acid and disodium phosphate increased the rate of color development. In these trials 0.075 mg. of furfural, 25 ml. of glacial acetic acid containing 2.5 ml. of aniline, and varying amounts of the other reagents were used. The final volume was 50 ml. and the relative color intensities of the solutions were determined by means of a Kuder photoelectric colorimeter.

Table I. Effect of Oxalic Acid and Disodium Phosphate on Color of Furfural Solutions

Sample No.	Treatment		Kuder Readings[a] 30 min.	45 min.	1 hour	1.25 hours
	Grams					
1	1.0	NaCl	47.5	48.4	48.5	47.5
2	0.50	Na_2HPO_4	48.7	48.8	47.5	46.0
3	0.25	Na_2HPO_4	48.2	48.1	46.8	44.9
4	0.12	Na_2HPO_4	48.4	48.2	46.8	44.6
5	0.5 0.12	Na_2HPO_4 Oxalic acid	49.1	50.2	50.1	49.4
6	0.25 0.12	Na_2HPO_4 Oxalic acid	49.2	50.2	50.2	49.5
7	0.12 0.12	Na_2HPO_4 Oxalic acid	48.5	50.2	50.1	49.8
8	0.50 0.12	NaCl Oxalic acid		Cloudy		
9	0.25 0.25 0.12	NaCl Na_2HPO_4 Oxalic acid	48.9	50.0	50.7	50.2

[a] All values represent means of from 2 to 4 determinations. Kuder readings are directly proportional to color intensity of solutions.

The results given in Table I show that the addition of 0.12 to 0.50 gram of disodium phosphate to the color reagents increases the rate of color development, but did not improve the stability of the color. The addition of a mixture of 0.12 gram of oxalic acid and 0.12 to 0.5 gram of disodium phosphate increased the intensity of color as compared to the sodium chloride treatment, and the solutions were more stable than those containing disodium phosphate alone. The addition of 0.25 gram of sodium chloride to this mixture had little or no effect on the color. A mixture of oxalic acid and sodium chloride gave a cloudy solution after standing 30 to 45 minutes.

Twelve per cent hydrochloric acid is commonly employed in the distillation of pentoses, pentosans, etc., for the separation of furfural. Since Youngburg recommended phosphoric acid, it was decided to compare phosphoric and different concentrations of hydrochloric acid.

A xylose solution containing 0.25 gram of xylose per 25 ml. was used, and 25-ml. aliquots were placed in 500-ml. two-necked, round-bottomed flasks. Samples of arabinose, sweet clover, alfalfa, and crude hemicellulose were also distilled. Acids to give the following concentrations were added to the flasks: phosphoric acid, 57 per cent; hydrochloric acid, 12, 18.5, and 24 per cent. A steam inlet tube, which extended almost to the bottom of the flask, was placed through one opening, and the flask was connected through the other opening to a condenser by means of an adapter, provided with thermometer. All connections were interchangeable ground-glass joints. A steady stream of steam was passed into the flask, under which a small flame was placed. A vigorous boil was maintained throughout the distillation. The distillate was collected in 1-liter

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Table II. Determination of Furfural by Distilling in the Presence of Phosphoric and Hydrochloric Acids

Sample Material	Wt. of Sample	Acid Used H_3PO_4	Acid Used HCl	Temp. of Distn.	Time of Distn.	Furfural Found By titration	Furfural Found Colorimetrically
	Gram	%	%	° *C.*	*Hours*	%	%
Xylose	0.25	57	..	98–105	3	57.4, 57.4, 57.7	49.5, 49.5, 51.1
	0.25	57	..	110–12	2	55.3, 54.7	50.2, 51.0
l-Arabinose	0.25	57	..	110–12	1	44.4, 44.1, 44.7	37.0, 36.3, 36.9
Sweet clover	0.20	57	..	110–12	1	8.2, 8.2	5.1, 5.1
Alfalfa	0.20	57	..	110–12	1	9.3, 9.1	6.1, 6.1
Xylose	0.25	..	12	102–3	6	60.6, 60.8	55.1, 55.8, 54.9
	0.25	..	18.5	105	2.5	61.2, 61.0	54.8, 55.2, 54.8, 55.2
	0.25	..	24	105	2.5	60.8, 60.2	55.9, 54.8
Sweet clover	1.00	..	18.5	105	2.5	9.3, 9.0	6.0, 6.0, 5.9
Crude hemicellulose	0.10	..	18.5	105	2	33.2, 32.2	29.2, 29.2
Xylose	0.25	..	12	102–3	2	53.1	44.4
	0.25	..	12	102–3	3.5	58.0	51.1
	0.25	..	12	102–3	4.5	59.7	53.6
	0.25	..	12	102–3	6	60.8	54.9

volumetric flasks. The distillation is complete when several drops of the distillate placed in a test tube fail to give a color in 10 minutes when an equal volume of glacial acetic acid, containing 2.5 ml. of aniline per 25 ml., is added. The pH of the distillates was in the range of 3 to 5 and never failed to give a color test when furfural was present.

The furfural in the distillates was determined by both the titration procedure of Hughes and Acree (*1*) and the modified colorimetric method.

The results given in Table II show that higher furfural values were obtained when the distillations were carried out in the presence of hydrochloric acid as compared to phosphoric acid. Approximately equal values were obtained with 12, 18.5, and 24 per cent hydrochloric acid; however, 12 per cent hydrochloric acid required at least twice as long for the complete removal of furfural from the sample. The 18.5 and 24 per cent concentrations required about an equal length of time.

The titration procedure gave consistently higher values than the colorimetric procedure, but the reason for this was not clear. In an attempt to clarify this point it was decided to compare the results obtained by titration and colorimetrically when other sugars were distilled with 18.5 per cent hydrochloric acid. In addition, samples of xylose were reinforced with certain aldehydes and other compounds and distilled, and pure furfural was determined colorimetrically and by titration.

Table III. Titration and Colorimetric Values

[Distillates from rhamnose (methylpentose), levulose (hydroxymethylpentose), xylose, and xylose reinforced with 0.20 gram of aldehydes, etc.]

Substance Distilled	Wt. of Sample	Results Calculated as Furfural By titration	Results Calculated as Furfural Colorimetrically
	Gram	%	%
Rhamnose	0.17	29.6	0
Levulose	0.25	5.3	0
Xylose	0.25	58.3	55.6
Xylose + benzaldehyde	0.25	56.6	55.2
Xylose + vanillin	0.25	109.3	55.7
Xylose + quercitin	0.25	56.6	54.7
Xylose + salicylaldehyde	0.25	204.9	55.2
Furfural	1.000	98.7	98.7

Table III shows that methylpentose and hydroxymethylpentose gave a titration value but did not produce a color with the furfural color reagents. When xylose was reinforced with certain aldehydes the distillates gave higher titration values than xylose alone. The colorimetric procedure gave no higher values. Quercitin and benzaldehyde had no apparent effect on either the colorimetric or the titration procedure. Pure furfural gave almost theoretical values when titrated or measured colorimetrically. Samples of pure furfural (not shown) gave approximately 100 per cent recovery by steam-distillation. These results indicate that the colorimetric method is more accurate for the determination of furfural. Since plant materials probably produce certain reducing substances on distillation with acids, these might interfere with the bromate titration method, as pointed out by other investigators.

To obtain evidence as to the effect of substances distilled from alfalfa and sweet clover on the colorimetric method, spectrophotometric curves of the color produced from the distillates of alfalfa and sweet clover were compared with similar curves plotted from pure furfural color solutions of equal concentration. Within experimental error all the curves were similar, which indicated that no appreciable interfering substances were present in the alfalfa or sweet clover distillates.

Procedure

A sample of pentose, pentosan, or plant material having a furfural content between 1 and 15 mg. is placed in a 500-ml. two-necked round-bottomed flask. Hydrochloric acid and water in sufficient amount to give between 200 and 225 ml. of about 18.5 per cent hydrochloric acid (approximately 1 to 1 by volume) is added to the sample. A steam tube extending almost to the bottom of the flask is inserted through one opening, and the flask is connected to a condenser through an adapter containing a thermometer. A steady stream of steam is passed into the solution and a flame is placed under the flask. When the contents of the flask begin to boil vigorously the flame is adjusted to hold the temperature between 105° and 106° C., measured at the opening into the condenser. The distillate is allowed to pass through a small filter, as it comes from the condenser, and then into a 1-liter volumetric flask. If the liquid in the distilling flask is reduced to approximately 75 ml., the steam inlet tube is removed and hydrochloric acid (1 + 1) is added to bring the volume back to 200 to 225 ml. The distillation is usually complete in 2 or 2.5 hours. This is determined when a few drops of the distillate fail to give a color in 10 minutes with an equal volume of glacial acetic acid containing 2.5 ml. of aniline per 25 ml. of acid.

The solution is diluted to the mark, and mixed, and 10-ml. aliquots are pipetted into 50-ml. volumetric flasks. The solutions are made just alkaline to phenolphthalein with sodium hydroxide, and 5 ml. of a solution containing approximately 0.12 gram of oxalic acid and 0.25 gram of disodium phosphate per 5 ml. are added. The solution is diluted to approximately 25 ml. with distilled water and 25 ml. of glacial acetic acid containing 2.5 ml. of freshly distilled aniline are added. The acetic acid and aniline should be mixed and allowed to come to room temperature just before using. It is then diluted to the 50-ml. mark, mixed, placed in a water bath at 20° C., and covered so that almost all the light is excluded. After 45 minutes to 1 hour it is read in a photoelectric colorimeter. The concentration of furfural corresponding to a given colorimeter reading is obtained by reference to a calibration curve made by plotting a series of known furfural solutions against colorimeter readings. If a photoelectric colorimeter is not available, the unknown solution may be compared to known standards in any type of colorimeter in general use.

Summary and Conclusions

The steam-distillation of pentoses and plant material with hydrochloric acid resulted in higher yields of furfural than steam-distillation with phosphoric acid. The use of 12 per cent hydrochloric acid required at least twice as long as 18.5 or 24 per cent hydrochloric acid to remove all the furfural. The yield of furfural from xylose was approximately the same when the three concentrations were used.

In the application of the aniline acetate colorimetric method, the addition of small amounts of oxalic acid and di-

sodium phosphate to the color reagents resulted in increased color intensity and shortened slightly the time required for maximum color to develop. The colorimetric method was not affected by methyl furfural or hydroxymethyl furfural. The distillates from alfalfa or sweet clover produced colored solutions very similar to that produced with pure furfural, as shown by spectrophotometric curves. The titration method of Hughes and Acree (*1*) gave consistently higher results than the colorimetric method when applied to the distillates from pure sugars, alfalfa, or sweet clover. This may be due to the presence of reducing substances other than furfural in the distillates. For these reasons it is thought that the colorimetric method may be more accurate.

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Determination of Total Sulfur in Rubber and Rubberlike Materials

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A modification is presented of the oxidation procedure employed in the standard method for the determination of total sulfur in rubber and similar materials. The method is especially valuable for the analysis of vulcanized synthetic rubberlike materials. It is likewise applicable to a wide variety of sulfur-containing organic compounds.

THE determination of total or combined sulfur in vulcanized rubber and materials of similar type involves numerous difficulties. This fact is attested by the very large number of methods which have been proposed (*3*).

The most important methods all involve oxidation of the sulfur, in whatever form it may be present, to sulfate and weighing the latter as barium sulfate. The only essential differences lie in the methods of oxidation employed.

The methods which seem to be the most popular at the present time are (*a*) oxidation of the sample in a Parr bomb with sodium peroxide, potassium chlorate, and sugar (*5, 6, 8, 9*); (*b*) oxidation with perchloric acid and nitric acid (*12*), sometimes with the additional use of bromine (*4*); (*c*) oxidation with nitric acid and bromine, followed by sodium carbonate–potassium nitrate fusion (*1, 2, 11*); and (*d*) oxidation with nitric acid–zinc oxide mixture, bromine, and potassium chlorate (*1, 2, 7*).

Serious explosions have been known to occur with perchloric acid oxidations; hence this method is not so popular at present as the other three. The fusion methods are reliable, but they are time-consuming and therefore not convenient when large numbers of samples must be handled.

Methods (*c*) and (*d*) have been for a number of years the preferred procedures of the A. S. T. M. For several years the fusion method was the "recommended" one and method (*d*), commonly referred to as the "Kratz-Flower" method, was listed as the "alternate" method (*1*). In 1940 (*2*) the order of listing was reversed, with the Kratz-Flower method becoming the recommended and, at least inferentially, the preferred procedure.

This procedure involves a preliminary oxidation with nitric acid and bromine, following which the mixture is slowly evaporated to a foamy sirup. Then, if organic matter or carbon remains at this point, a few milliliters of fuming nitric acid and a few crystals of potassium chlorate are added and the solution is evaporated at a boil. The operation is repeated until all carbon is gone and the solution is clear, colorless, or light yellow. It is then evaporated and nitrates are decomposed by one of two alternate methods.

The analyst is cautioned to use care during the addition of potassium chlorate. This is a standard precaution, to be found in all analytical textbooks. That it is well founded is proved by the fact that explosions frequently occur when the addition is carried out in this manner.

An additional disadvantage of this standard procedure is the fact that the seemingly drastic conditions of oxidation are still not sufficient to effect the oxidation of many samples. Carbon black is oxidized in some samples, but not in others. Some of the synthetic rubbers prove especially troublesome, as the solution reaches a dark, nearly black color which is not destroyed by repeated treatment.

A somewhat unorthodox modification of the above procedure has been found to take care of these difficulties and to yield a clear, light-colored solution with considerably less trouble. The writer does not claim to have originated the method; in its present form it is an adaptation of a general procedure with which he came in contact a number of years ago and the exact origin of which is not known. In the meantime, the method has been extensively tested, in the hands of both experienced analysts and students who were completely unfamiliar with the procedure. A number of variables affecting the procedure have thus been studied in some detail, and the operating details as indicated here are believed to be general in their application.

Modified Method

In the standard method as modified, the nitric acid–bromine oxidation is carried out as usual. Then the resulting solution is evaporated slowly until no more nitrogen oxide or bromine fumes are visible, diluted with 10 to 15 ml. of concentrated nitric acid, and heated to boiling. To the vigorously boiling solution are added successively several 0.5-gram portions of potassium chlorate.

Contrary to what one might expect, no explosion occurs. This procedure has been employed in hundreds of analyses of

Table I. Typical Sulfur Determinations

Sample	Theoretical	Parr Bomb	Modified A. S. T. M.
Rubber stock A	...	2.42	2.50
		2.45	2.45
Rubber stock B	...	1.80	1.80
		1.85	1.84
Rubber stock C	...	3.05	3.10
		3.09	3.14
Hycar O. R. stock	...	2.76	2.80
		2.80	2.81
Buna S stock A	...	1.76	1.78
		1.80	1.78
Buna S stock B	...	1.42	1.37
		1.40	1.42
Thiokol stock	...	47.20	47.30
		47.38	47.42
Mercaptobenzothiazole	38.42	38.28	38.50
		38.42	38.40
Ethyl-*p*-toluenesulfonate	15.98	16.00	16.00
		15.98	15.95
Thiocarbanilide	14.04	14.17	14.10
		14.12	14.17
n-Butyl sulfite	16.48	16.32	16.40
		16.37	16.47

various types of rubber products and explosions have occurred only where the solution was allowed to cool and was no longer boiling vigorously.

Carbon black is oxidized readily by this method, and the dark-colored solutions obtained from vulcanized synthetic rubber samples are readily decolorized to yield usually straw-colored solutions. If the successive portions of chlorate are added too rapidly, foaming may result. An occasional sample foams when companion samples do not; this variance may be due to the presence of certain compounding ingredients. A little practice will enable the analyst to anticipate cases of bad foaming, and to control them by quickly removing from the heat during the early stages of foaming, and, if necessary, cooling under tap water.

The exact amount of chlorate necessary to effect the oxidation varies with the individual sample. This is particularly true of those containing carbon black. The relative ease of oxidation seems to be related to the type of black. In general, any unnecessary excess of chlorate should be avoided, to prevent contamination of the barium sulfate precipitate by other salts. A large excess of chlorate definitely tends to favor co-precipitation.

Since potassium chlorate is somewhat difficult to obtain in war time, a number of determinations have been made with an analytical grade of sodium chlorate. The sodium salt may be substituted for the potassium with no noticeable loss in efficiency, but where both are available, the potassium salt is preferred, as it can usually be obtained in a higher state of purity.

Following the chlorate treatment the normal procedure may be employed, with no additional modifications.

This general method for the determination of sulfur is also adapted for the analysis of various sulfur-containing compounds, especially organic ones. It is reasonably convenient and merits more attention than it has received in the past from those engaged in analyzing materials other than rubber. It has been used successfully by the writer in analyzing a wide variety of sulfur compounds; none of those investigated have failed to be oxidized by this method. However, it is not a completely universal method, and some judgment must be exercised in its use. In the case of long-chain organic sulfur compounds of very considerable molecular weight, and relatively high sulfur content (*10*), the recurrence of units in the chain of the type —RSR— is common and on oxidation can give rise to sulfones of such stability that even the sodium peroxide fusion method very frequently leads to low results. The only method that gives consistently accurate results in the case of this type of compound involves the practically instantaneous oxidation of the sample in a bomb under about 30 atmospheres of oxygen pressure.

Many organic compounds are completely oxidized by the nitric acid–bromine combinations and the chlorate is then unnecessary.

In Table I are listed a few of the more typical results obtained by this procedure. The Parr bomb fusion with sodium peroxide was chosen as a reference method for these samples. The agreement between these two methods is good and the analytical results for the pure compounds check well with the calculated values.

Complete Procedure

Place 0.5 gram of soft rubber or 0.2 gram of hard rubber in a 500-ml. Erlenmeyer flask of chemically resistant material (Pyrex, quartz, etc.), add 10 ml. of zinc oxide–nitric acid solution (containing 200 grams of zinc oxide to 1000 ml. of nitric acid, specific gravity 1.42), and moisten the sample thoroughly. Let stand at least 1 hour, overnight if convenient. The sample becomes partly decomposed; this permits the addition of fuming nitric acid with no danger of ignition of the sample. Add 15 ml. of fuming nitric acid and whirl the flask rapidly to keep the sample immersed to avoid ignition. With some samples it may be necessary to cool the flask under running water.

When the solution of the rubber appears to be complete, add 5 ml. of a saturated water solution of bromine and slowly evaporate the mixture until no more fumes of nitrogen oxide or bromine are visible. Dilute the solution with 10 to 15 ml. of concentrated nitric acid and heat to boiling. To the vigorously boiling solution add about 0.5 gram of potassium chlorate (or an analytical grade of sodium chlorate). If the solution is still dark in color, continue to boil and add another portion of chlorate. Continue the addition of successive portions of chlorate until all carbon is gone and the solution is clear, colorless, or light yellow. Do not use an excess of chlorate over the amount required to complete the oxidation. (Caution: Foaming may result if the portions of chlorate employed are too large or if the successive portions are added too rapidly.) If foaming starts, cool immediately, if necessary under running water.

Evaporate the mixture to dryness and bake at the highest temperature of a Tirrill burner until all nitrates are decomposed and no more nitrogen oxide fumes can be detected. Anneal the flask carefully to prevent cracking.

Add 50 ml. of hydrochloric acid (1 to 6) to the cooled flask and digest warm until solution is as complete as possible. Filter the solution, wash the filter, and dilute the solution to 300 ml. Add 10 ml. of saturated picric acid solution, precipitate with 10 per cent barium chloride, and allow the precipitate to stand overnight. Filter, wash with hot water until the filter paper is colorless, and determine the barium sulfate precipitate in the usual manner.

Acknowledgment

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Importance, Composition, and Analysis of Bolivian Tin Concentrates

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IT IS characteristic of all strategic metals, minerals, and ores that their domestic supply is inadequate and that there exists a marked inequality of distribution among producing and consuming countries. This is particularly true in the case of tin. This country, the world's largest consumer of tin (almost 50 per cent of the world output) is compelled, with the exception of some secondary recovery, to import all its tin from distant countries.

Prior to the present war, approximately 70 per cent of the total world production of tin ore, mostly from placer deposits, was produced in southeastern Asia (Malaya, China, Dutch East Indies) and about 20 per cent, mostly from lode deposits, in Bolivia.

Repeated attempts have been made to exploit small and low-grade deposits in this country, but there seems little hope that an appreciable output of tin ore can be developed, as known deposits are too small in size and too low in grade to justify work on a scale that would produce an output of appreciable magnitude (*14*).

Until recently, the smelting picture was not much brighter. The smelters in Malaya and the Dutch East Indies used to produce more than 60 per cent of the world's metallic tin; Bolivia, the world's second largest ore producer, does no smelting; and England, which is only a small ore producer (2 per cent of the world production), is the second largest smelter.

There have been only two noteworthy attempts in the past to operate smelters in the United States (with Bolivian ores), one in 1907, the other in 1916; both ended in utter failure, chiefly because of political and commercial control of the ore supplies. As tin is probably the most important strategic metal, Roush advised (*15*) a reserve stock pile containing enough metal to tide over anticipated needs for one or two years.

Therefore, early in the present crisis, the Government increased imports of tin from abroad and stepped up secondary recovery, particularly from tin plate scrap and very recently from used tin cans. In addition, and despite failures in the past and views of pessimistic observers, the Government, represented by the Metals Reserve Company, concluded, as early as November, 1940, five-year contracts with a number of Bolivian tin ore producers, providing for the annual delivery of many thousand tons of tin ores and concentrates. A new and impressive tin smelter has been built in Texas and is operated for the U. S. Government by the Dutch N. V. Billiton subsidiary, called the Tin Processing Corporation, a group which reportedly has had "more actual experience in smelting refractory Bolivian ores than any other available person or corporation" (*2*). It is hoped that the operation of this vital plant will go a great way toward establishing a permanent tin industry in this country. Since production and supply of tin are essential factors contributing to victory, the importance of the Bolivian tin concentrates (although of lower quality than the Malayan and East Indian concentrates) cannot be overestimated, particularly in the light of recent military events in the Far East.

Table I. Composition of Bolivian Tin Concentrates

Grade	Sn	Pb	Cu	Sb	As	Bi	Zn	S	Fe	SiO_2	WO_3	Ti
	%	%	%	%	%	%	%	%	%	%	%	%
High	64.15	0.02	0.38	0.10	0.49	0.003	0.19	2.17	[a]	[a]	[a]	[a]
High	57.78	1.14	0.52	0.78	0.45	0.012	0.38	2.53	7.69	4.32	0.06	0.14
High	59.30	0.32	0.62	0.09	0.53	0.004	2.47	4.13	6.42	4.70	0.17	0.03
High	62.19	0.02	0.17	0.18	0.27	0.027	0.21	2.35	[a]	[a]	[a]	[a]
Medium	38.20	0.09	0.32	0.18	0.14	0.033	0.78	3.13	[a]	[a]	[a]	[a]
Medium	52.83	1.38	0.07	0.65	0.62	0.008	4.14	4.79	[a]	[a]	[a]	[a]
Medium	48.92	0.14	0.87	0.64	0.22	0.008	0.07	3.33	7.17	19.14	[a]	[a]
Medium	36.49	4.17	0.66	0.14	0.46	0.230	10.09	12.99	[a]	[a]	[a]	[a]
Low	19.07	0.33	0.54	0.57	0.58	0.131	0.24	2.63	[a]	[a]	[a]	[a]
Low	24.16	1.13	0.03	0.14	0.34	0.026	23.12	24.55	15.68	[a]	[a]	[a]
Low	22.16	0.92	0.96	3.48	0.56	0.074	0.37	8.19	19.57	22.91	0.75	1.21
Low	23.11	0.75	2.11	0.34	0.07	0.217	0.03	6.77	12.15	38.00	3.07	[a]

[a] Not determined.

Composition of Bolivian Tin Concentrates

The chemical analysis of the Bolivian tin concentrates presented a major problem to the laboratories entrusted with this task, as these concentrates are of widely varying grade and composition and the amount of impurities ranges from zero to many per cent.

The tin concentrates are of the cassiterite (SnO_2) type, stannite ($Cu_2S.FeS.SnS_2$) and thealite ($PbS.SnS_2$) being rejected by terms of the contract. They are delivered in 3 grades: high grade, over 55 per cent tin; medium grade, over 35 per cent tin; low grade, over 18 per cent tin.

Bolivian tin concentrates are contaminated by varying amounts of silica, iron, lead, copper, bismuth, antimony, arsenic, and zinc, mostly in the form of oxides and sulfides. Sometimes concentrates containing varying amounts of tungsten, titanium, aluminum, and calcium are encountered, as well as manganese, cadmium, molybdenum, vanadium, tantalum, columbium, silver, and indium, mostly in very small quantities. The contracts between the Metals Reserve Company and the Bolivian tin producers provide that more than small but defined amounts of lead, copper, bismuth, antimony, arsenic, zinc, and sulfur are objectionable, certain penalties and treatment charges being imposed for their presence. Some typical composition figures obtained in actual analysis (Table I) indicate some of the difficulties that analysts encounter in judging the approximate composition of the tin concentrates, and are helpful in choosing the proper analytical procedure to be followed.

As the writer has been engaged in the analysis of tin concentrates, he decided to test all methods mentioned in the literature that appeared to be suitable, to determine concisely their range of application, and to compile and present them in a form that may prove helpful not only to laboratories engaged in analysis of these particular concentrates, but also to chemists performing assays on similar material.

In some instances, a procedure incorporating the best features of several methods has been developed, which gives reliable results and is generally applicable to almost any

type of tin concentrate. In other cases, however, new suggestions and procedures had to be worked out and these have been included in the investigation reported here. Spectrochemical methods for the determination of impurities in tin concentrates are becoming more popular as methods are being perfected. One laboratory engaged in the analysis of Bolivian tin concentrates first identifies all samples spectroscopically, and unless the amount of the impurity exceeds a certain limit, it is quantitatively determined by spectrochemical analysis. The high cost of spectroscopic equipment, however, and its limited range of application for routine quantitative analysis caused this writer to omit a detailed description of such methods.

Preparation and Solution of Sample

Cassiterite is scarcely attacked by any acid, but some of the impurities present in the form of sulfide or oxide (arsenic, zinc, sulfur, copper) can be dissolved by proper acid treatment.

Other methods, applied in the procedures described below, are:

1. Fusion with a mixture of sodium peroxide and sodium carbonate in a nickel or iron crucible. The writer has carried out numerous tests to determine the most efficient and economical fusion mixture. Sodium peroxide alone, used by the majority of laboratories, though very effective with cassiterite and other ores, is extremely corrosive and readily attacks nickel and iron crucibles, frequently causing loss of money and time when fusions "melt through". However, a mixture of 1 part of sodium carbonate with 2 parts of sodium peroxide not only produces perfect decomposition of the sample but lessens the corrosive action of the sodium peroxide upon the crucible, considerably lengthening its life.
2. Cassiterite can be decomposed by fusion with a mixture of equal parts of sodium carbonate or potassium carbonate and sulfur. Tin, antimony, and arsenic (also any molybdenum, tungsten, and vanadium) are found in the water extract of the fusion, while iron, zinc, copper, lead, and bismuth (as well as any titanium, manganese, and cadmium) appear in the residue. This method of decomposing tin concentrates, however, should be avoided when the sample is high in iron or zinc.
3. Cassiterite is reduced to tin by ignition in a stream of hydrogen, most of the impurities also being found in the metallic residue. Fusion of the concentrate with potassium cyanide and other reducing fluxes will effect a similar reduction to the metal.
4. Fusion of cassiterite with certain oxidizing lead fluxes will result in its decomposition, tin and impurities passing into the slag and bismuth being collected in the lead button.

Determination of Tin (Routine Method)

Weigh 1 gram of low-grade tin or 0.5 gram of medium- or high-grade tin concentrate into a 50-ml. high-form iron or nickel crucible, add 4 grams of sodium carbonate, mix thoroughly, and cover with 8 grams of sodium peroxide. Heat the crucible and contents over a small Bunsen flame to expel any water in the flux and until the charge starts melting, then carefully finish fusion by holding and revolving the crucible around the outer edge of the flame until the mixture melts down quietly. When the contents are in quiet fusion, continue heating with the full flame of the Bunsen burner, while rotating the crucible, for about 2 minutes.

Let the melt solidify and cool, then place the crucible in a 400-ml. beaker, add 60 ml. of cold water, and allow the melt to disintegrate. Remove the crucible from the beaker with a glass rod, rinsing it thoroughly with warm water. Add 50 ml. of concentrated hydrochloric acid to the beaker, agitating the solution until all has dissolved, as indicated by the clear green color of nickel chloride or the brown color of ferric chloride. Clean the crucible with 50 ml. of hydrochloric acid and add to main solution.

Transfer the solution to a 500-ml. Erlenmeyer flask and dilute to 250 ml. Introduce a nickel strip or foil weighing about 5 grams and boil the solution gently for about 25 minutes until all ferric chloride has been reduced (if fusion of the sample was carried out in an iron crucible). Introduce a second nickel strip and close the flask with a rubber stopper containing a glass tube extending on the outside to the bottom of the flask. Boil the solution gently for 20 more minutes, then seal the end of the glass tube with a hot solution of sodium bicarbonate in a 250-ml. beaker, remove the flask from the hot plate, cool to below 15° C., and titrate with a 0.125 N iodine solution, using starch as indicator.

Strength of Iodine Solution. Weigh 160 grams of c. p. iodine and 320 grams of c. p. potassium iodide into a large beaker and cover with distilled water. Allow to stand for several days with occasional stirring, adding more water until all is dissolved, filter through asbestos into a dark bottle, and dilute with distilled water to approximately 10 liters. Allow to stand for about 2 weeks. The strength of the iodine solution is such that all low-grade tin concentrates (tin <35 per cent) and all high-grade concentrates (tin <78 per cent) can be run on the 1.0-gram or 0.5-gram basis, respectively, using in either case less than 50 ml. of the iodine solution. It is undesirable to reduce and titrate more than about 400 mg. of tin.

Standardization of Iodine Solution. 1. As stannous chloride is very unstable and the titer of the iodine solution changes somewhat with the concentration of acid and salts, it is best to use one of the standard tin concentrates made available by the National Bureau of Standards and fuse, reduce, and titrate it, as described above, simultaneously with the sample which is analyzed.

2. If a standard sample of a similar composition is not at hand, it may be replaced by finely rolled and cut tin foil, which is easily fused and decomposed in a nickel or iron crucible by the same fusion flux ($Na_2CO_3 + Na_2O_2$) used for cassiterite.

3. Adding to the hydrochloric acid solution of standard tin a "blank fusion" of sodium peroxide in an iron crucible, in order to introduce the same amount of salts and approximate conditions prevailing during the reduction of the solution of the sample, is a standardization procedure sufficient for routine work where extreme accuracy is not required but is inadequate when highly accurate results are expected. The sample has to undergo a number of manipulations during which it is subject to possible mechanical losses by spattering, volatilization, etc. On the other hand, by dissolving tin in hydrochloric acid and adding a blank fusion, the standard is not submitted to the same manipulations and possible mechanical losses as the sample. Consequently this standardization procedure produces somewhat low tin results.

4. One laboratory proposes the use of a "standard solder" of known tin content, which is dissolved in nitric acid (1 gram of the solder). Metastannic acid thus obtained is filtered off, ignited, and fused exactly like the sample. The writer believes that this standardization procedure is objectionable, not only as it raises the question of the reliability of the "standard solder", but also because any chemical or mechanical loss of metastannic acid would raise the titer of the iodine solution. The tendency of this procedure is to give high tin results.

Nature of Interference. Few of the elements found in Bolivian tin concentrates will interfere with the above method.

Most of the arsenic is expelled as trichloride, and most of the antimony precipitated by the nickel foil. Small amounts of arsenious and antimonious compounds are without effect if the solution contains sufficient acid. Larger amounts of antimony (>50 mg.) will obscure the end point and are best removed by treating the hydrochloric acid solution with iron nails and subsequently filtering it prior to the reduction with nickel. Small quantities of copper (<50 mg.) do not interfere as they are precipitated by the nickel, but larger amounts should be removed with iron. The small amounts of bismuth found in Bolivian tin concentrates are precipitated by the nickel and do not interfere. Tungsten is reduced to a lower oxide by the nickel, presumably to the blue tungsten pentoxide, which, however, does not consume any iodine. When much tungsten is present the blue color thus produced is apt to obscure the end point of the starch indicator. In such cases lead should be used instead of nickel to reduce the stannic chloride.

There are contradictory statements in the literature concerning the exact nature of interference of molybdenum, Mantell (*11*) asserting that molybdenum has no effect, while Scott (*16*) claims that it is reduced by the nickel (presumably to the trichloride) and is subsequently reoxidized by the iodine. Tests carried out by the writer would support this latter view. The reoxidation of the molybdenum trichloride, however, is not proportional to the amount of molybdenum present. According to Scott (*16*) reduction

with lead will obviate the above difficulties. Vanadium is also reduced by the nickel and partly reoxidized by the iodine solution, and in the presence of large amounts of vanadium reduction should be carried out with lead. Titanium chloride is reduced to titanous chloride by the nickel. The reduced compound, however, is oxidized only by an excess of iodine, and even then the action is extremely slow.

Modifications of the above method have been proposed.

Some analysts use iron, antimony, zinc, and aluminum instead of nickel, to reduce the stannic chloride. The method favored by many European laboratories (*4*) consists in removing copper, antimony, etc., by reducing the hydrochloric acid solution of the tin with "ferrum reductum" and subsequently filtering into a 750-ml. Erlenmeyer. The solution is then treated with aluminum, heated until both the aluminum and tin have dissolved, cooled, and titrated, after starch has been added, with a solution of iodine or of ferric chloride, using in the latter case an indicator of potassium iodide and cuprous iodide. During the entire procedure the air in the Erlenmeyer flask is replaced by carbon dioxide gas. The ferric chloride solution is made up by dissolving 144 grams of $FeCl_3.6H_2O$ or 86 grams of $FeCl_3$ in 300 ml. of concentrated hydrochloric acid and 1000 ml. of water. The solution is then diluted to 3000 ml. The indicator used for this titration is prepared by adding 50 grams of hydriodic acid (specific gravity 1.50) to 100 grams of potassium iodide and 25 grams of cuprous iodide. Next 50 ml. of warm water are added and the solution is stirred until the cuprous iodide has dissolved and then decanted into a stock bottle containing some copper wire. To the undissolved potassium iodide 50 ml. of hydriodic acid and 50 ml. of warm water are added and after all has dissolved this is added to the main solution. Carbon dioxide is passed in until the solution is colorless.

Potassium Iodate Method

As pointed out above, the chief difficulty in obtaining satisfactory tin results is that the reduced compound is extremely unstable and tends to reoxidize in contact with air.

Because of this instability of stannous chloride, most analysts try to reduce the time of titration (unless special precautions, such as eliminating admission of air by passing carbon dioxide over the surface of the solution, are employed during the titration) by running the assay with a small portion of the sample (0.25 to 0.5 gram) or by using for the titration a comparatively strong iodine solution, 1 ml. of which titrates 10 mg. of tin.

Both procedures will meet routine requirements, but are not adequate if extremely accurate results are expected. For, if the assay is run with 0.5 or 0.25 gram of the sample, any plus or minus error in the manipulation will be doubled or quadrupled in the final calculation. On the other hand, if the analysis is carried out with 1 gram of the sample and titration is performed with an iodine solution, 1 ml. of which oxidizes 10 mg. of tin, in order to obtain extremely accurate results an agreement of 0.1 ml. or less in the titration must be reached.

A method, recently worked out by this writer and described below, successfully obviates the difficulties attending the usual procedures. It is not intended for routine work, as the approximate tin content of the sample must be known or first be determined by another method. However, it gives excellent results and makes it possible to take for one tin determination 2 grams or more of high-grade cassiterite. The procedure was originally intended and may be highly recommended for determination of tin in pig tin and tin-bearing alloys by dissolving 2 to 5 grams of the sample in hot concentrated sulfuric acid and proceeding as described below.

Procedure (Example, N. E. I. tin concentrate, tin ≐ 75 per cent). Weigh 2-gram portions of tin concentrate, and 2 grams of a standard sample containing approximately the same amount of tin, into nickel crucibles and fuse with 5 grams of sodium carbonate and 10 grams of sodium peroxide. Acidify, reduce, and cool along the lines indicated in the regular iodine method, described above, using a 750-ml. Erlenmeyer flask.

Weigh in 0.8800-gram portions of potassium iodate if the sample contains 75 to 78 per cent of tin, or 0.8500-gram portions of potassium iodate if the sample contains 73 per cent of tin, add about 0.5 gram of sodium bicarbonate and about 1 gram of potassium iodide, and dissolve in 75 ml. of cold water. (As 0.9 gram of potassium iodate oxidizes approximately 1.50 grams of tin, the amount of potassium iodate weighed in as directed above is sufficient to oxidize approximately 96 to 97 per cent of the stannous chloride present. 0.6010 gram of KIO_3 ≐ 1.0000 gram of tin.)

Remove the stopper with the glass tube from the Erlenmeyer flask containing the reduced tin solution and add at once quantitatively the potassium iodate solution, washing the beaker with cold water. Add starch solution and titrate immediately with a potassium iodate solution, 1 ml. of which contains 0.0030 gram of potassium iodate.

Prepare the potassium iodate solution by dissolving 3.0000 grams of potassium iodate and about 3 grams of sodium bicarbonate in cold water. Transfer to a 1000-ml. volumetric flask, fill up to the mark, and shake.

Calculation

Standard Sample Containing 75.93% Sn		N. E. I. Tin Concentrate	
KIO_3 added, gram	0.8800		0.8800
Iodate titrated, ml.	12.2		11.0
	12.3		11.0
			10.95
12.25 ml. of KIO_3 ≐ 36.75 mg. of KIO_3		10.99 ml. of KIO_3 ≐ 32.97 mg. of KIO_3	
0.8800 gram of KIO_3		0.8800 gram of KIO_3	
0.03675 gram of KIO_3		0.03297 gram of KIO_3	
0.91675 gram of KIO_3		0.91297 gram of KIO_3	

$$\text{Sample contains } 75.93 \times \frac{0.91297}{0.91675} = 75.62\ \%\ \text{Sn}$$

Notes. 1. If a standard sample is not at hand, standardization may be carried out by fusing 1.5000 grams of finely cut c. p. tin foil in a nickel crucible with sodium carbonate and sodium peroxide.

2. As the oxidation value of the potassium iodate differs only very slightly from its theoretical titer, it is adequate for routine analysis of samples of approximately known tin content to establish an empirical factor.

3. Instead of the potassium iodate solution a weak (0.075 *N*) iodine solution may be used to carry out the end titration. To carry out the standardization of this iodine solution, a certain measured amount of this solution and a certain weight of potassium iodate (which is dissolved in water and acidified with hydrochloric acid, and to which some potassium iodide solution is added) are both titrated with an approximately 0.1 *N* sodium thiosulfate solution. Thus the strength of 1 ml. of the iodine solution and also the amount which was required to carry out the end titration can be expressed in grams of potassium iodate. This weight, in turn, is added to the weight of potassium iodate which originally had been added to oxidize the bulk of the stannous chloride.

Determination of Copper, Lead, and Antimony

The determination of copper in cassiterite has been the cause of some controversy between the laboratories involved, as one side has insisted that methods based on acid decomposition of the sample, although rapid and easy of manipulation, are in most cases insufficient and will invariably produce low copper results, while the other side insists that acid decomposition is adequate.

The writer supports the former view and bases this opinion on an extensive investigation which is recorded in Table II.

In 2 series of tests copper was thus determined in a large number of samples both by the two regular methods (described

Table II. Reliability of Acid Decomposition

Type of Tin Concentrate	Copper Found by Acid Decomposition		Copper Found by Sulfur Soda Fusion		Copper Found by Na_2O_2 Fusion	
	Original	Duplicate	Original	Duplicate	Original	Duplicate
	%	%	%	%	%	%
High grade	0.57	0.58	0.69	0.70	0.71	0.69
	0.18	0.20	0.36	0.34	0.37	0.36
	0.08	0.08	0.08	0.08	0.09	0.07
	0.43	0.44	0.57	0.59	0.60	0.58
Medium grade	0.72	0.69	0.91	0.89	0.90	0.90
	0.35	0.35	0.40	0.42	0.43	0.40
	1.19	1.23	1.44	1.46	1.45	1.43
	0.68	0.64	0.71	0.69	0.68	0.68
Low grade	0.75	0.76	0.94	0.95	0.88	0.90
	0.24	0.27	....	....	0.33	0.33
	2.21	2.18	2.37	2.38	2.39	2.40
	1.53	1.48	1.57	1.60	1.61	1.61

below) and by decomposing the sample with aqua regia and sulfuric acid. The results definitely show that acid decomposition is not sufficient and will not give the full copper content. However, duplicate results obtained by this method are generally in good agreement, thus misleading some analysts to the belief that they are reliable.

Warmbrunn (*3*) arrived at a similar conclusion, although the material that he analyzed was somewhat different.

Method I. This method is applicable to all grades of cassiterite, although Method II, described below, has advantages under certain defined conditions.

Procedure. Weigh 1.0 gram of cassiterite into a 50-ml. iron crucible, mix with 4 grams of sodium carbonate, cover with 8 grams of sodium peroxide, and fuse as for tin. Transfer the crucible, when cool, to a 400-ml. beaker, add 100 ml. of water, and allow the melt to disintegrate. Remove the crucible and wash with hot water. Add just enough hydrochloric acid to give a clear solution, but disregard undissolved iron scale. Rinse the crucible with dilute hydrochloric acid and add to main solution. Pass in sulfur dioxide for about 5 minutes to reduce the ferric chloride, then expel excess sulfur dioxide by gentle boiling. Neutralize the warm (but not hot) solution carefully with about 6 *N* ammonia until a permanent deep green precipitate forms, then add 6 *N* hydrochloric acid drop by drop until the precipitate turns white, and finally an excess of 6 ml. of the same acid. Dilute to 250 ml., pass in hydrogen sulfide for about 20 minutes, dilute to 325 ml., and continue passing in hydrogen sulfide for 10 more minutes.

Allow the precipitate to settle, filter on 12.5-cm. No. 40 Whatman filter paper, and wash 6 times with warm 1 per cent sulfuric acid wash solution containing hydrogen sulfide. Wash the precipitate back into the original beaker and place the latter under a funnel. Pour 60 ml. of potassium sulfide solution (prepared by dissolving 75 grams of potassium hydroxide and 20 grams of sodium sulfate in 500 ml. of water, saturating the solution with hydrogen sulfide, and diluting to 1000 ml.) over paper. Warm on a hot plate until the soluble portions have been extracted. Filter through the original paper into a 500-ml. Erlenmeyer flask and wash with warm dilute potassium sulfide solution. Save the filtrate for the antimony determination.

Place the precipitate and paper in the original beaker and fume with 20 ml. of nitric acid and 8 ml. of sulfuric acid, adding, if necessary, more nitric acid to destroy all carbonaceous compounds. Finally fume strongly to expel excess nitric acid. Dilute the cool solution with 75 ml. of water and heat to boiling. Cool the solution for 2 hours to below 15° C., filter off lead sulfate on a 9-cm. No. 42 Whatman filter paper, and wash 5 times with cold one per cent sulfuric acid. Save filtrate for the copper determination.

Wash the lead sulfate back into the original beaker and place the latter under the funnel. Pour 30 ml. of ammonium acetate solution (300 grams of the salt in 1000 ml. of solution) over paper. Warm on a hot plate until lead sulfate has dissolved, then filter through the original paper, receiving the filtrate in a 250-ml. beaker and washing with hot dilute ammonium acetate solution. Add 5 ml. of 12 *N* hydrochloric acid and heat to boiling. (The addition of hydrochloric acid instead of acetic acid tends to prevent the precipitation of basic lead chromate upon the addition of ammonium chromate.) Add to the boiling solution 20 ml. of 20 per cent ammonium chromate solution and continue with the boiling until the precipitate has turned to a shade of orange. Filter on a tared Gooch crucible, dry at 105° C., and weigh as lead chromate.

To the filtrate of the lead sulfate add 10 ml. of 50 per cent tartaric acid and make the solution slightly ammoniacal, then slightly acid with hydrochloric acid. Add 20 ml. of strong sulfur dioxide water and 2 ml. of 10 per cent ammonium thiocyanate solution. Stir the solution vigorously to accelerate coagulation of copper thiocyanate, filter on 11-cm. No. 42 Whatman paper, and wash the precipitate once with cold water. Place the precipitate in the original beaker and take to fumes with nitric acid and 5 ml. of sulfuric acid until all the carbonaceous compounds are destroyed and heavy fumes of sulfur trioxide escape. Dilute with 300 ml. of water, add 5 ml. of ammonia and 3 ml. of nitric acid, and determine the copper electrolytically.

Acidify the potassium sulfide extract containing the antimony with 75 ml. of 9 *N* sulfuric acid, warm until the precipitate has settled, and filter on 12-cm. No. 40 Whatman paper, washing the precipitate with dilute sulfuric acid containing hydrogen sulfide. Place the precipitate and paper in the original Erlenmeyer flask. Take to fumes with 1 gram of potassium bisulfate, 3 grams of ammonium sulfate, and 30 ml. of 36 *N* sulfuric acid by heating, first cautiously, then with the full heat, on a very hot plate until all carbon is destroyed.

Add to the cool solution 75 ml. of strong sulfur dioxide water and 100 ml. of 12 *N* hydrochloric acid and boil down on a hot plate to 85 ml. to expel all arsenic as trichloride. Dilute with 250 ml. of water, cool to below 15° C., and titrate with 0.05 *N* potassium permanganate solution which has been standardized with c. p. antimony.

If titration with potassium bromate is preferred, add 120 ml. instead of 100 ml. of 12 *N* hydrochloric acid, boil down to a volume of about 100 ml., add 100 ml. of hot water, and titrate with 0.02 *N* potassium bromate solution, using methyl orange as indicator.

Method II. This method is very rapid, accurate, and well suited for the determination of lead and copper in high-grade cassiterite. Considerable difficulties are, however, encountered when applying it to low-grade concentrates high in iron, and, to a lesser degree, to those concentrates high in zinc. This is due to the tendency of iron sulfide to enter into soluble complex compounds with sodium sulfide, while the filtration of zinc sulfide from alkaline solution is difficult. This method is also well adapted for the determination of antimony in cassiterite provided that the amount of the latter does not exceed a few milligrams. With a higher antimony content slightly low results will be obtained with this procedure, and preference should be given to Method I.

Procedure. Weigh 1 gram of cassiterite into a 150-ml. tall-form porcelain beaker, mix thoroughly with 4 grams of sodium carbonate and 5 grams of sulfur, and cover with 3 grams of potassium carbonate. Heat the beaker with the full flame of a Bunsen burner until the charge has melted down quietly, keeping the beaker covered with a watch glass. Allow the melt to cool, then add 3 grams of sodium sulfite and 120 ml. of water and heat on a hot plate to effect disintegration of the melt and extraction of the soluble portions. Filter on 11-cm. No. 40 Whatman filter paper, receiving the filtrate in a 500-ml. Erlenmeyer flask and washing the precipitate with warm dilute potassium sulfide solution. Proceed with the antimony determination as in Method I. For the determination of lead and copper, place the precipitate in a 250-ml. beaker, and clean the porcelain beaker with a little nitric

Table III. Accuracy of Methods I and II

Type of Tin Concentrate	Found by Method I			Found by Method II			Reputed Pb, Cu, Sb Content		
	Pb	Cu	Sb	Pb	Cu	Sb	Pb	Cu	Sb
	%	%	%	%	%	%	%	%	%
High grade	0.41	0.17	0.42	0.44	0.19	0.40	0.40	0.15[a]	0.38
	1.14	0.52	0.78	1.17	0.51	0.73	1.18	0.40[a]	0.73
	0.02	0.17	0.18	0.05	0.17	0.20	0.06	0.15[a]	0.19
	0.14	0.08	0.09	0.14	0.09	0.12	0.10	0.09[a]	0.10
Medium grade	0.09	0.32	0.18	0.12	0.29	0.15	0.12	0.23[a]	0.14
	1.38	0.07	0.65	1.36	0.09	0.62	1.32	0.08[a]	0.61
	0.14	0.87	0.64	0.15	0.88	0.61	0.15	0.66[a]	0.60
	0.23	0.45	0.02	0.20	0.49	0.00	0.25	0.36[a]	0.00
Low grade	0.75	2.11	0.34	0.73	2.15	0.29	0.77	1.98[a]	0.36
	0.92	0.96	3.48	0.93	0.99	3.38	0.97	0.86[a]	3.43
	0.68	0.36	0.63	0.64	0.40	0.59	0.71	0.30[a]	0.59

[a] Copper determined by acid decomposition.

acid which is then added to the 250-ml. beaker. Add 20 ml. of 16 *N* nitric acid and 8 ml. of 36 *N* sulfuric acid, and proceed as in Method I.

Some results of actual copper, lead, and antimony determinations are given in Table III.

Determination of Arsenic

Weigh 1.0 gram of cassiterite into a 250-ml. beaker, add 20 ml. of 16 *N* nitric acid and 15 ml. of 36 *N* sulfuric acid, and evaporate slowly on a hot plate to strong fumes of sulfur trioxide. When cool, add 15 ml. of strong sulfur dioxide water and take again to fumes, repeating the evaporation once more with 10 ml. of distilled water.

Transfer the solution to a distilling flask, using the least amount of water possible. Add 45 ml. of hydrazine sulfate solution (10 grams of hydrazine sulfate and 20 grams of potassium bromide in 1000 ml. of solution) and 70 ml. of concentrated hydrochloric acid. Immerse the outlet of the condenser beneath the surface of 250 ml. of cold water in a 600 ml. beaker. Distill until the volume in the flask has been reduced to 75 ml., then add 40 ml. more of hydrochloric acid and distill again to 75 ml.

Remove the beaker from under the condenser, washing the latter with distilled water and allowing washings to run into the distillate. Make the solution alkaline with ammonia, using methyl orange as indicator, then just acid with hydrochloric acid. Cool to below 15° C., add 8 grams of sodium bicarbonate, and titrate with 0.03 *N* iodine solution, using starch as indicator.

Standardize the iodine solution by dissolving a weighed amount of arsenic trioxide in a little sodium hydroxide (0.5 to 1 gram) and 10 ml. of water, dilute to 250 ml., add 100 ml. of concentrated hydrochloric acid, neutralize with ammonia, acidify with hydrochloric acid, cool, and proceed with the titration as for the sample.

Table IV. Application Range of Two Zinc Methods

Type of Tin Concentrate	Reputed Zinc Content	Zinc Found by Method I		Zinc Found by Method II	
		Original	Duplicate	Original	Duplicate
	%	%	%	%	%
High grade	...	0.10	0.12	0.09	0.13
	...	0.70	0.70	0.73	0.70
	2.47	2.49	2.45	2.44	2.47
Medium grade	1.12	1.12	1.09	1.10	1.08
	4.73	4.76	4.78	4.73	4.75
	2.47	2.40	2.45	2.50	2.46
Low grade	0.99	0.99	0.98	0.99	0.96
	12.89	12.81	12.78	12.91	12.88
	16.64	16.52	16.50	16.62	16.64
	23.98	23.88	23.88	24.00	23.96

Determination of Zinc

In contrast to copper which is found chemically combined with cassiterite and other tin ores (stannite!), zinc seems not to have entered into such combinations, but merely accompanies the other impurities of tin concentrates. Hence, there have been very few instances where the writer in determining zinc in tin concentrates has had cause to distrust results obtained by Method I which is based on acid decomposition. This procedure, which is very rapid and accurate, can be used as long as the amount of zinc does not materially exceed 10 per cent. Method II should be preferred for tin ores of poor quality which occasionally contain up to 25 per cent of zinc, although this method is more lengthy and not so easy of manipulation as Method I. Preference should also be given to Method II whenever doubts arise as to whether or not acid decomposition is sufficient. Results which indicate the range of application of both methods are given in Table IV.

Method I. Weigh 2.0 grams of cassiterite into a 250-ml. beaker, add 25 ml. of 16 *N* nitric acid and 15 ml. of sulfuric acid, and evaporate on hot plate until heavy fumes of sulfur trioxide escape. Take up the cold solution with 100 ml. of water, add 3 grams of ammonium sulfate, and heat to boiling. Pass in hydrogen sulfide for about 5 minutes to precipitate arsenic, copper, antimony, bismuth, etc., and the small amounts of tin which have been brought into solution. (If the sulfide precipitate is large, indicating that the sample consists largely of stannite or other tin sulfides, it is advisable to discard this procedure and start another portion of the sample using Method II.) Filter on 9-cm. No. 2 Whatman filter paper and wash the precipitate 5 times with warm 3 per cent sulfuric acid containing hydrogen sulfide. Receive the filtrate in a 400-ml. beaker, heat to boiling to expel hydrogen sulfide, then oxidize the solution with hydrogen peroxide. Add 12 *N* ammonia until the solution is strongly alkaline. Heat to boiling, filter off ferric hydroxide on 12-cm. No. 2 Whatman filter paper, receiving the filtrate in a 600-ml. beaker, and wash 4 times with hot water. Wash the precipitate back into the original beaker and redissolve iron hydroxide in a little sulfuric acid and hydrogen peroxide. Repeat the ammonia separation, filter through the original paper into the original filtrate, and wash the precipitate 4 times with hot water. Discard the precipitate.

Table V. Determination of Zinc

Zinc Found as Zinc Oxide	Zinc Found Electrolytically	Zinc Found by Titration
%	%	%
0.38	0.34	...
0.64	0.59	...
0.80	0.76	0.73
1.29	1.22	1.20
3.77	3.66	3.70
5.12	5.02	5.00
8.83	8.72	8.74
13.01	....	12.89
18.38	....	18.24
24.27	....	24.14

Evaporate the filtrate to approximately 250 ml., cool to below 20° C., add 3 drops of methyl orange, then 9 *N* sulfuric acid, drop by drop, until the yellow color just turns red, then add 2 drops of the same acid in excess. Pass in hydrogen sulfide for half an hour, allow zinc sulfide to settle, filter on 11-cm., No. 42 Whatman paper, and wash the precipitate 3 times with warm water. Ignite zinc sulfide and paper in a clay or porcelain crucible, first at a low heat, then with the full flame of a Bunsen burner, and for 5 minutes with a blast lamp, raising the heat to approximately 900° C. Cool in a desiccator and weigh as zinc oxide.

The results are usually slightly high, owing to incomplete oxidation of the sulfide. Hence it is advisable to verify the results by one of the two following procedures:

Procedure for Less Than 100 Mg. of Zinc. This procedure is based on the electrolytic deposition of zinc from sodium hydroxide solution, a method that is not particularly in vogue in this country, even though extremely accurate, if properly executed.

Transfer the zinc oxide to a 250-ml. beaker, add 15 ml. of 18 *N* sulfuric acid, and evaporate to fumes of sulfuric acid. Take the solution up with 100 ml. of water and add solid sodium hydroxide until just alkaline, then about 4 grams in excess. Filter off, if necessary, any iron hydroxide, cadmium hydroxide, or sulfur present, through a small paper into a 250-ml. electrolytic beaker and wash the paper with hot water containing some sodium hydroxide. Dilute to approximately 250 ml. and electrolyze the zinc from this solution with 2 volts and 0.5 ampere on a platinum cathode which has previously been coated with not more than 20 mg. of copper.

Titration with Potassium Ferrocyanide. Transfer the zinc oxide to 600-ml. beaker and dissolve in 15 ml. of 6 *N* hydrochloric acid. Add 5 grams of ammonium chloride and dilute to 250 ml. Pass in hydrogen sulfide for 2 minutes, heat the solution to boiling, and titrate with a weak solution of potassium ferrocyanide (9 grams of $K_4Fe(CN)_6$ in 1000 ml. of solution), using uranyl acetate as an external indicator. Standardize with C. P. zinc.

Results obtained by these two procedures are usually in good agreement, although slightly lower than those calculated from the weight of the zinc oxide (Table V).

Method II. Method II is more time-consuming and its execution more exacting than Method I. Its application should therefore be confined to tin ores high in zinc or containing acid-insoluble zinc.

Procedure. Fuse 1.0 gram of cassiterite in a nickel crucible, as for tin. Place the cool crucible in a 400-ml. beaker and add 100 ml. of water and 60 ml. of concentrated hydrochloric acid. Remove the crucible and rinse with dilute hydrochloric acid. Heat

the solution to boiling to expel chlorine, then adjust the acidity to 25 ml. of hydrochloric acid in 250 ml. of solution. Pass in hydrogen sulfide for 15 minutes, dilute to 350 ml., and continue passing in hydrogen sulfide for 10 more minutes.

Allow the precipitate to settle, filter on 11-cm., No. 40 Whatman filter paper, receiving the filtrate in a 600-ml. beaker, and wash 5 times with warm 3 per cent sulfuric acid containing hydrogen sulfide. Wash the precipitate back into the original beaker, add 10 ml. of hydrochloric acid and 3 grams of potassium chlorate, and heat on a hot plate until the sulfides have dissolved and chlorine has been expelled. Dilute to 100 ml., pass in hydrogen sulfide for 15 minutes, dilute to 150 ml., and continue passing hydrogen sulfide for 15 more minutes. Let the precipitate settle and filter through the original paper into the original filtrate containing the bulk of the zinc. Wash the precipitate 4 times with 3 per cent sulfuric containing hydrogen sulfide. Discard the precipitate.

Heat the filtrate to boiling to expel hydrogen sulfide, then oxidize with hydrogen peroxide and proceed as in Method I.

Note. If the final zinc sulfide precipitate appears discolored, indicating that some nickel sulfide had been coprecipitated, dissolve the zinc sulfide in hot dilute hydrochloric acid, add 2 ml. of 36 *N* sulfuric acid, and evaporate to fumes of sulfur trioxide. Take the solution up with 200 ml. of water and render it just alkaline with ammonia, using methyl orange as indicator. Then render it acid with 9 *N* sulfuric acid, adding an excess of 2 drops of the latter. Pass in hydrogen sulfide for half an hour and proceed as in Method I.

Table VI. Influence of Tin

Tin Present	Expected Weight of $BaSO_4$	Actual Weight of $BaSO_4$
Gram	*Grams*	*Grams*
0.24	0.3284	0.3254
0.37	0.2917	0.2911
0.26	0.7426	0.7405
0.26	1.6893	1.6878
0.55	0.3645	0.3629

Determination of Sulfur

Good sulfur results are frequently the product of a number of compensating plus and minus errors. For instance, many analysts (*6*, *13*), find that the plus error due to coprecipitation of barium chloride and the minus error caused by the solubility of barium sulfate in water generally balance each other, if some precautions are observed. Mellon (*12*) speaks of entrainment of barium chloride as a minus error when precipitating barium sulfate by adding sulfuric acid to a solution containing barium chloride.

There are, however, a number of other factors which may largely influence the determination of sulfur in tin ores, necessitating more or less tedious purification or correction procedures. Some of the more important factors causing low sulfur results are:

1. Precipitation of barium sulfate in the presence of sodium, potassium, and ammonium chloride causes coprecipitation of sodium, potassium, and ammonium sulfate, instead of barium sulfate; these are of lower molecular weight, hence producing low sulfur results. In the presence of ammonium chloride this minus error is greater, because of the volatility of ammonium sulfate.
2. Precipitation of barium sulfate in the presence of the same salts also causes coprecipitation of bisulfates of sodium, potassium, and ammonium, of lower molecular weight than barium sulfate, and decomposition at high temperatures, changing first into the pyrosulfates and then into the normal sulfates. In the case of ammonium chloride this minus error is greatest because of the volatility of all ammonium sulfate salts.

$$2NaHSO_4 \rightarrow Na_2S_2O_7 + H_2O$$
$$Na_2S_2O_7 \rightarrow Na_2SO_4 + SO_3$$

3. Precipitation of barium sulfate in the presence of ferric salts causes coprecipitation of ferric sulfate, of lower molecular weight than barium sulfate, and decomposition into ferric oxide and sulfur at 480° C.
4. Precipitation of barium sulfate in the presence of nitrates causes entrainment of barium nitrate by the barium sulfate, producing high sulfur results. This tendency is lessened, eliminated, or even reversed in the presence of alkali or ammonium salts, because of their tendency to produce low sulfur results.
5. Precipitation of barium sulfate in the presence of even large amounts of tin will produce satisfactory sulfur results (Table VI).

With the above cited defects and sources of error in mind, let us consider the pros and cons of those procedures that seem to have possibilities for the determination of sulfur in tin ores and concentrates.

Method I. Fusion with Sodium Peroxide. Fusion of tin ores requiring sodium peroxide as flux eliminates the use of platinum crucibles and necessitates the employment of nickel crucibles, thus introducing large amounts of nickel salts, which are without any influence upon the precipitation of barium sulfate.

Procedure. Weigh 1.0 gram of tin concentrate into a nickel crucible and fuse as for tin. When the fusion is cool, place the crucible in a 600-ml. beaker, add 75 ml. of water, and allow the melt to disintegrate. Add 70 ml. of hydrochloric acid and agitate until all is in solution. Remove the crucible and wash it with dilute hydrochloric acid. Evaporate the solution to dryness on a water bath or hot plate and dehydrate the silica by heating the residue for half an hour at about 105° C. Add 5 ml. of concentrated hydrochloric acid and 200 ml. of water, heat to boiling, filter on 11 cm. No. 42 Whatman filter paper, receiving the filtrate in a 600-ml. beaker, and wash with hot water. (Hold the precipitate for possible test for barium sulfate.)

Dilute the solution to 500 ml., heat to boiling, then add slowly with the help of a pipet or a precipitating cup (*8*) enough 10 per cent barium chloride solution to precipitate the sulfate ion and provide an excess of about 5 ml. Allow barium sulfate to settle overnight and filter on 11-cm. No. 42 filter paper. Wash the precipitate 3 times by decantation with hot water, transfer it to the paper, and continue the washing until the test for chloride ion fails. Dry the wet paper in a clay or porcelain crucible and ignite carefully in a good oxidizing atmosphere, finally raising the heat to about 900° C. Cool in a desiccator and weigh as barium sulfate.

In order to demonstrate the application range of this method and its remarkable merits, unfortunately restricted, however, to certain types of samples, it has been applied in the analysis of a large number of tin ores of reputed sulfur content. Minus errors due to coprecipitation of sodium sulfate and sodium bisulfate (*7*), ferric sulfate and tin sulfate were accurately determined (Table VII).

Table VII. Accuracy of Method I, Based on Fusion

Reputed Sulfur Content	Sulfur Found, Uncorrected	Sulfur Found, Corrected	Na_2SO_4	$NaHSO_4$	$Fe_2(SO_4)_3$	$Sn(SO_4)_2$
%	%	%	+%	+%	+%	+%
2.11	2.08	2.13	0.02	0.01	0.01	0.01
2.87	2.83	2.89	0.02	0.02	0.02	..
3.46	3.39	3.46	0.02	0.02	0.02	0.01
4.55	4.47	4.57	0.03	0.02	0.04	0.01
7.68	7.56	7.67	0.03	0.02	0.04	0.02
10.24	10.10	10.26	0.05	0.04	0.04	0.03
13.72	10.55	10.74	0.06	0.06	0.04	0.03
18.93	18.66	18.95	0.11	0.10	0.05	0.03
23.37	23.02	23.38	0.16	0.13	0.04	0.03

The data in Table VII clearly indicate that Method I, although subject to many sources of error, gives excellent results as long as the sulfur content does not exceed a certain percentage (5 to 8 per cent). As the uncorrected sulfur figures of samples in this category deviate only slightly from the true sulfur content, and the split agreed upon by the Metals Reserve Company and the Bolivian tin processors, is 0.25 per cent, it would represent a major waste of time to execute the corrections mentioned above for every sulfur determination.

On the other hand it is apparent that with increasing amounts of sulfur the reliability of this method undergoes

Table VIII. Accuracy of Method II

Sulfur Found, Method I (Corrected)	Sulfur Found, Method II	Sulfur Found in Residue of Method II
%	%	%
2.13	2.02	0.09
2.89	2.73	0.11
3.46	3.28	0.15
4.57	4.39	0.14
7.67	7.49	0.15
10.26	10.02	0.18
13.74	13.50	0.18
18.65	18.39	0.20
23.38	23.12	0.22

a marked decline. For tin ores containing as much as 25 per cent of sulfur, there is scarcely any certainty that results obtained by this method represent the true sulfur content, even if all plus and minus errors have been corrected. One source of a minus error, coprecipitation of ferric sulfate, could be eliminated by prior reduction of the ferric to the ferrous ion with aluminum, zinc, or iron, since ferrous sulfate is not coprecipitated with barium sulfate. As this procedure would eliminate only one source of error and still would necessitate corrections of the remaining discrepancies, it seems safer, more accurate, and more economical to have recourse to a correction procedure, similar to that proposed by Hillebrand and Lundell (*8*). A correction graph is prepared, based on the analysis of a great number of tin ores of known sulfur content, covering the whole range of the regular samples, by preparing the solution containing the sulfate ion and precipitating the barium sulfate under exactly the same conditions and in the presence of the same impurities as in the regular routine samples.

Method II. Sulfur Determination Based on Acid Decomposition. A survey of the methods for the determination of sulfur, in use at the present time in various laboratories engaged in the analysis of tin ores and concentrates, disclosed that the majority of chemists prefer methods based on acid decomposition of the sample, frankly admitting, however, various discrepancies and uncertainties encountered in the execution of all such procedures. Procedures based on acid decomposition more or less follow the line of operation used for pyrite samples (*1*, *17*).

Procedure. Treat 1 gram of the sample either with a mixture of bromine and carbon tetrachloride followed by nitric and hydrochloric acids (*1*), or with a mixture of 3 parts of nitric acid and 1 part of hydrochloric acid containing a little bromine (*17*). Subsequently evaporate the solution on the water bath to dryness, add hydrochloric acid, and repeat the evaporation. Dehydrate silica by heating at approximately 100° C., take up the dried mass in 5 ml. of concentrated hydrochloric acid, and warm until solution of the soluble parts is complete, then treat with aluminum or zinc until colorless, filter, and wash the precipitate with hot water. Dilute the solution to about 500 ml., heat to boiling, and precipitate barium sulfate as usual.

In order to determine the accuracy and application range of this method, it was applied by the writer to the determination of sulfur in a great number of tin ores of known sulfur content; it became apparent that these methods in their original form are incompatible with accuracy, mostly because of incomplete decomposition of the sample and failure to recover the sulfur in the undecomposed residue (Table VIII).

Methods for the determination of sulfur in pyrites and other sulfides, based on acid decomposition of the sample, usually result in complete decomposition of the sample, leaving very little, if any, undecomposed residue. But this is different in the case of most tin ores, and particularly with cassiterite. The preponderance of stannic oxide causes partial envelopment of the sulfide particles, with incomplete decomposition of the sample, and thus very often produces irregular, erratic, and always low sulfur results; hence it is imperative to determine sulfur also in the residue from acid decomposition. This, however, involves lengthy procedures. As ignition of the filter paper containing the undecomposed residue would cause certain loss of the sulfide sulfur, one of the two following procedures may be used:

1. Wash the residue into a nickel crucible, evaporate to dryness on a water bath, mix with sodium carbonate and sodium peroxide, and proceed as in Method I, which is based on immediate fusion of the sample.
2. Dry the paper and residue for 3 hours in an electric oven at approximately 70° C., brush the residue into a nickel crucible, and proceed as in Method I.

Method III. The writer recently succeeded after many fruitless attempts and with the defects of the above methods in mind, in developing a method, also based on acid decomposition, which is easy of manipulation and gives good results (Table IX).

It is based on the strong oxidizing power of 70 per cent perchloric acid at its comparatively high boiling point of approximately 200° C. Preliminary treatment of the sample prior to the addition of perchloric acid consists in oxidizing the bulk of the sulfide sulfur to the sulfate ion by a mixture of bromine and acetic acid, followed by nitric and hydrochloric acids. The excess of the acids is expelled by perchloric acid, which in its turn readily attacks the residue, oxidizing any remaining sulfur to the sulfate ion. To prevent a loss of sulfuric acid during this evaporation, a little calcium nitrate is added (*5*).

The new procedure has been applied to all types of tin concentrates, regardless of their sulfur content, and is very rapid, as it does not involve corrections of plus and minus errors or the preparation of a correction graph as in Method I or the lengthy treatment of the residue as in Method II.

Procedure. Weigh 1.0 gram of tin ore into a 250-ml. beaker, add 10 ml. of a mixture of 3 parts of acetic acid and 1 part of bromine (each 100 ml. of which contain 10 grams of calcium nitrate or an equivalent amount of zinc nitrate), and add 3 ml. of bromine. Allow to stand at room temperature for about 15 minutes, then add 8 ml. of nitric acid and 8 ml. of hydrochloric acid and warm gently until action ceases. Add 15 ml. of perchloric acid and evaporate slowly on a hot plate to fumes of perchloric acid, then heat strongly for about 3 minutes. Allow the solution to cool, add 120 ml. of water, and 10 ml. of concentrated hydrochloric acid, and heat to boiling. Reduce with zinc or aluminum until the solution is colorless. Filter on 11-cm. No. 42 Whatman filter paper into a 600-ml. beaker and wash with hot water. Dilute the filtrate to about 500 ml., heat to boiling, and add 10 per cent barium chloride solution with the help of a pipet or a precipitating cup. Determine and weigh as barium sulfate.

Table IX. Accuracy of Method III

Sulfur Found, Method I (Corrected Results)	Sulfur Found, Method III Original	Sulfur Found, Method III Duplicate
%	%	%
2.13	2.10	2.14
3.46	3.47	3.44
10.26	10.22	10.23
23.37	23.40	23.42

Determination of Bismuth

Bolivian tin concentrates generally are low in bismuth, most of them containing less than 0.03 per cent. In some instances, however, bismuth was found to be as high as 0.5 per cent.

The dissolving of samples with such small amounts of bismuth is rather a difficult task, as acid decomposition is insufficient, fusion with a mixture of sodium carbonate and sulfur causes great losses of bismuth, and fusion with sodium

peroxide in an iron or nickel crucible is confined to the opening of small portions of cassiterite. The following procedure enables the handling of 5 to 10 grams of tin concentrate by collecting the bismuth by lead in a furnace fusion, while tin and the remaining impurities pass into the slag.

Procedure. Weigh 5 grams of high-grade concentrates or 10 grams of medium- or low-grade concentrates into 30-gram clay crucibles, containing 75 grams of flux No. 1 (No. 1 flux consists of: litharge 15,000, pearl ash 1000, soda ash 2500, borax glass 1500, silica 500, flour 500), mix thoroughly, and add 75 grams of flux No. 2 (No. 2 flux consists of: litharge 10,000, pearl ash 3000, soda ash 2000, borax glass 5400, silica 2000, argols 800), cover with borax glass, and fuse in a muffle furnace. When fusion is complete, remove from the furnace and pour into an iron mold. When cool, clean the lead button by hammering, add enough test lead to give 65 grams (combine at this point two or more lead buttons if the sample is extremely low in bismuth), place in 7.5-cm. (3-inch) scorifiers, and scorify down to a button weighing between 18 and 25 grams.

To continue, modifications of two reliable procedures may be used.

Method I. Ammonium Formate Method (*9*). Flatten the lead button, place in a 600-ml. beaker, add 200 ml. of water, and 45 ml. of concentrated nitric acid, and warm to complete solution. Neutralize the warm solution with 8 *N* ammonia until further addition would cause precipitation of lead and bismuth. The solution should be definitely clear at this point, but the acidity should not exceed 2 ml. of 16 *N* nitric acid in 300 ml. of solution. Add 8 ml. of 40 per cent ammonium formate solution. Allow to stand warm for 1 hour, when as little as 1 mg. of bismuth has settled. Filter off the precipitate on 11-cm. No. 42 Whatman paper and wash 8 times with hot water. Dissolve the precipitate in warm 4 *N* nitric acid, evaporate to dryness on a water bath, and take up with 1 ml. of hydrochloric acid (1 to 9), 2 grams of ammonium chloride, and 5 ml. of hot water. Warm to complete solution, then dilute to approximately 500 ml. and allow bismuth oxychloride to settle. Filter and wash with hot water. Dry at approximately 105° C. and weigh as bismuth oxychloride.

If only minute amounts of bismuth are present, dissolve the bismuth formate in hot (6 *N*) sulfuric acid and determine bismuth colorimetrically with potassium iodide.

Method II. Ledoux's Method (*10*). Dissolve the lead button in nitric acid as in the preceding method. Add carefully to the warm solution 8 *N* ammonia until the excess acid is neutralized and a faint cloud but no permanent precipitate forms. Now add 1 ml. of hydrochloric acid (1 to 9). The solution will clear for a moment, then bismuth, when present in appreciable amounts, will precipitate and coagulate rapidly as a white crystalline precipitate. Allow it to settle on the hot plate until the supernatant solution appears clear. Filter on No. 42 Whatman filter paper and wash with hot water. Dissolve the precipitate, which contains only small amounts of lead, in hot dilute nitric acid and proceed as in Method I.

Other Impurities

A complete discussion of methods dealing with the determination of other impurities is not attempted. A short outline of the more important determinations follows.

Determination of Silica. Fuse 1 gram of cassiterite in a nickel crucible with sodium carbonate and sodium peroxide. Leach with water in a nickel dish. Pour the solution into a casserole or a beaker containing enough hydrochloric acid to render the solution acid. Evaporate to dryness and dehydrate the silica by heating at 105° C. Take up with 10 ml. of hydrochloric acid and 200 ml. of water. Heat to boiling, filter off silica, ignite in a platinum crucible, weigh, expel the silica with hydrofluoric acid and a drop of sulfuric acid, reignite, and weigh.

Determination of Tungsten. Fuse 1 gram of the sample as for silica. Transfer the crucible to a 600-ml. beaker and leach fusion with water. Acidify with hydrochloric acid and evaporate the solution to dryness, then take it up with 10 ml. of hydrochloric acid and 200 ml. of warm water, and heat it to boiling. Add 5 ml. of cinchonine solution, and allow to stand overnight. Filter off the precipitate and wash with 10 per cent cold hydrochloric acid. Wash the precipitate back into the original beaker, which is then placed under the funnel. Pour 40 ml. of 6 *N* ammonia over the paper, warm the solution on a hot plate, filter through the original paper into a 400-ml. beaker, and wash with 10 per cent ammonia. Dilute the solution to 200 ml., and expel ammonia by boiling, acidify with 5 ml. of hydrochloric acid, dilute to 200 ml., add 5 ml. of cinchonine solution, and allow to stand overnight. Filter, wash the precipitate with dilute cinchonine solution, ignite in a tared platinum crucible, expel the silica with hydrofluoric acid and a drop of sulfuric acid, reignite, and weigh as tungsten trioxide.

Determination of Iron, Aluminum, Titanium, Calcium, and Magnesium. Use the filtrate from the silica determination or fuse 1 gram of the sample in a nickel crucible with sodium carbonate and sodium peroxide. Leach the fusion with 100 ml. of water in a 400-ml. beaker, add 65 ml. of concentrated hydrochloric acid, remove the crucible, and rinse it with dilute hydrochloric acid. Heat the solution to expel chlorine, then adjust acidity to 15 ml. of hydrochloric acid in 300 ml. of solution. Pass in hydrogen sulfide for half an hour and allow the precipitate to settle. Filter, receiving the filtrate in a 600-ml. beaker and washing the precipitate with warm 3 per cent sulfuric acid containing some hydrogen sulfide. Discard the precipitate, expel the hydrogen sulfide by boiling, then oxidize the solution with hydrogen peroxide.

Render the solution ammoniacal, heat to boiling, filter off, precipitate, and wash with 5 per cent ammonia containing some ammonium chloride. Dissolve precipitate in hydrochloric acid and repeat the ammonia separation, filtering into the main filtrate.

In the filtrate calcium may be precipitated as the oxalate and in the filtrate of the calcium oxalate the magnesium as magnesium ammonium phosphate.

Dissolve the precipitate containing the iron, aluminum, etc., in dilute hydrochloric acid, adjust the acidity to 5 ml. of hydrochloric acid in 150 ml. of solution, add 2 grams of tartaric acid, and pass in hydrogen sulfide for 20 minutes. Filter off any precipitate and wash with 1 per cent sulfuric acid, containing hydrogen sulfide and a little tartaric acid. Discard the precipitate. Render the solution ammoniacal, adding an excess of 5 ml. of ammonia. Pass in hydrogen sulfide for 5 minutes, filter off precipitate immediately, and wash it with an ammonium sulfide solution containing tartaric acid.

Transfer precipitate to the original beaker and take to fumes with nitric and sulfuric acids until all carbonaceous matter has been destroyed. To expel any nitric acid, take the solution twice to fumes with water. Finally determine the iron by reducing it either with stannous chloride or by the Jones reductor and subsequent titration with potassium permanganate.

Boil the filtrate containing the titanium and aluminum to expel ammonium sulfide, add 40 ml. of hydrochloric acid and again boil to expel all hydrogen sulfide, add 30 ml. of hydrogen sulfide, and again boil to expel excess peroxide. Dilute to 300 ml. cool, add a 6 per cent cupferron solution until no further precipitate forms. Filter, using pulp, and wash with cold water containing 100 ml. of hydrochloric acid per liter. The precipitate contains the titanium which may be contaminated by zirconium (quantitatively) and by some columbium and tantalum. Ignite the precipitate in a platinum crucible, fuse with bisulfate, take up with 150 ml. of about 3 *N* sulfuric acid, add 5 ml. of 3 per cent hydrogen peroxide, and compare the color with that produced by a standard solution of titanium. If larger amounts of titanium are present than can be accurately determined by this colorimetric procedure, pass the solution through a Jones reductor, receive it in a solution of ferric sulfate, and titrate with potassium permanganate.

If zirconium has to be determined, it may be precipitated with diammonium phosphate in the presence of hydrogen peroxide after the colorimetric titanium determination.

Add 25 ml. of sulfuric acid and 40 ml. of nitric acid to the filtrate of the cupferron precipitate and boil down to incipient fumes on a hot plate, adding more nitric acid from time to time until all organic matter has been oxidized. Take the solution up with 150 ml. of water, heat to boiling, filter off any silica, and wash with dilute sulfuric acid. Precipitate aluminum in the filtrate with ammonia, filter off, and wash with hot water containing ammonium nitrate. If chromium and beryllium are absent, ignite the precipitate in a tared platinum crucible and finally weigh as aluminum oxide.

If chromium is present (indicated by the color of the precipitate), dissolve into a 400-ml. beaker with hot dilute hydrochloric acid (1 to 1), add 25 ml. of perchloric acid, and boil till the chromium appears to be oxidized, and then for 5 more minutes. (If a chromium determination is required, use dilute nitric acid, instead of hydrochloric acid, to dissolve the hydroxide precipitate.) Cool, take up with 200 ml. of water, and neutralize with ammonia until distinctly ammoniacal. Heat to boiling, filter (through asbestos, if a chromium determination is required), and wash with hot water containing ammonium nitrate. In the absence of

beryllium, ignite the precipitate in a tared platinum crucible and finally weigh as aluminum oxide. In the presence of beryllium, separate aluminum using 8-hydroxyquinoline.

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Determination of Iodate in the Presence of Bromate and Chlorate

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THE method of van der Meulen (*2*) for the volumetric determination of iodate in the presence of bromate, while accurate, involves a long and rather complicated procedure. It has been found that by carefully buffering the reaction mixture it is possible to determine iodate by means of an ordinary iodometric titration without interference from bromate or chlorate. After the titration of iodate, bromate can be titrated in the same mixture by adding an adequate amount of hydrochloric acid and a few drops of a molybdate solution as a catalyst (*1*).

Preliminary experiments indicated that the reaction between iodate and iodide was rapid and complete in phthalate buffers between pH 4 and 5. Under the same conditions the reaction between bromate and iodide is slow. At pH 5 it is slow enough to be disregarded during the time required for a titration unless the concentration is high. The rate of the reaction is more rapid at pH 4. By proper choice of buffer it is possible to have all the iodate present in a mixture react without appreciable interference from the bromate. The buffer added has two functions. It supplies the hydrogen ions necessary for the iodate-iodide reaction. It also adjusts the pH of the medium to a value at which the iodate reaction is rapid, whereas the bromate reaction is slow, though measurable. The pH of the buffered solution increases during the reaction between iodate and iodide, since hydrogen ions are used up. Thus the pH is raised to a value at which the rate of reaction between bromate and iodide is negligibly small.

Table I. Titration of Iodate

(Titration of 25.00-ml. samples of 0.1 N KIO_3 with 0.09791 N $Na_2S_2O_3$)

0.1 N $KBrO_3$ Added	Biphthalate	$Na_2S_2O_3$ Used[a]	Error
Ml.	*Grams*	*Ml.*	%
..	2	25.54	+0.04
..	2	25.54	+0.04
..	3	25.55	+0.08
..	3	25.53	0.00
..	1.5	25.53	0.00
..	1.5	25.54[b]	+0.04
25	2	25.55	+0.08
25	2	25.53	0.00
25	2	25.56	+0.08
25	6	25.60	+0.27
25	6	25.60	+0.27
[c]	2	25.71	+0.7
[c]	2	25.80	+1.1

[a] Theoretical 25.53.
[b] Stood 5 minutes before titration.
[c] 1 g. of $KBrO_3$ present.

Table II. Titrations of Iodate-Bromate Mixtures with 0.09713 N Sodium Thiosulfate

0.1 N KIO_3	0.1 N $KBrO_3$	Biphthalate	$Na_2S_2O_3$ Used	$Na_2S_2O_3$ Theoretical	Error
Ml.	*Ml.*	*Grams*	*Ml.*	*Ml.*	%
25	100	2.0	25.75[a]	25.74	+0.04
25	100	2.0	25.75[a]	25.74	+0.04
50	25	4.0	51.45	51.48	−0.06
50	25	4.0	51.48	51.48	0.00
5	25	2.0	5.210	5.148	+1.2
5	25	2.0	5.240	5.148	+1.9
5	25	0.5	5.153	5.148	+0.1
5	25	0.5	5.153	5.148	+0.1
5[b]	0	0.5	5.148	5.148	0.00
10[c]	0	1.0	10.30	10.30	0.00
10	10	1.0	10.30	10.30	0.00
10	10	1.0	10.30	10.30	0.00
25	0[d]	2.0	25.74	25.74	0.00
25	0[d]	2.0	25.74	25.74	0.00

[a] End point tended to drift slightly.
[b] 20 ml. of H_2O added.
[c] 10 ml. of H_2O added.
[d] 10 ml. of 1 N $KClO_3$ added.

Experimental

Aliquot portions of a standard 0.1 N potassium iodate stock solution were treated with various amounts of solid potassium biphthalate. Potassium iodide was added and the mixtures were titrated with standard sodium thiosulfate. In each case the major portion of the thiosulfate was added from a pipet and the titration was finished with a microburet. This technique

Table III. Titrations with 0.01 N Solutions in Presence of 1 Gram of Potassium Biphthalate

0.01 N KIO_3	0.1 N $KBrO_3$	0.009713 N $Na_2S_2O_3$	$Na_2S_2O_3$ Theoretical	Error
Ml.	*Ml.*	*Ml.*	*Ml.*	%
25	0	25.71	25.74	−0.12
25	0	25.70	25.74	−0.16
25	5	25.77	25.74	+0.12
25	5	25.77	25.74	+0.12
25	1	25.75	25.74	+0.04
25	0[a]	25.71	25.74	−0.12
25	0[a]	25.70	25.74	−0.16
10	1	10.34	10.30	+0.40
10	1	10.31	10.30	+0.10

[a] 2 ml. of 1.0 N $KClO_3$ present.

permitted reproducibility to about 0.05 per cent in titrations requiring approximately 25 ml. of solution. Titrations were made also with varying amounts of potassium bromate or chlorate present. The following procedure was found to give excellent results.

To 25 to 50 ml. of solution (containing about 2 to 3 milliequivalents of iodate) 2 grams of potassium biphthalate are added and the mixture is swirled until solution is effected. Three grams of potassium iodide are added and the mixture is allowed to stand for 3 minutes. The liberated iodine is then titrated with standard 0.1 N sodium thiosulfate to a starch end point.

The amount of biphthalate added supplies a threefold excess of hydrogen for the iodate-iodide reaction. The pH of a pure biphthalate solution is 4.0, but the reaction between iodate and iodide removes sufficient hydrogen ions to raise the pH to 5.0 (with 2.5 milliequivalents of iodate). This is important, as the iodate reaction thus takes place within a few seconds at the higher acidity and automatically reduces the hydrogen-ion concentration to a level at which the bromate reacts very slowly. If a much larger excess of biphthalate were to be added, a drifting end point and high results would be obtained.

Analytical Results

The data in Table I show that the conditions of the procedure permit an accurate titration of iodate and that the amount of biphthalate used is not critical. Tables I and II show that a fourfold excess of bromate or chlorate does not interfere.

The approximate ratio of 2 grams of biphthalate to 2.5 milliequivalents of iodate is necessary if results accurate to 0.1 per cent or better are desired. The above ratio of biphthalate to iodate is not critical, but large excess of biphthalate gives results accurate to only 0.25 or 2 per cent if bromate is present in equal or greater amounts. If the order of magnitude of the iodate concentration is not known, a preliminary titration permits estimation of the proper amount of biphthalate to be added for the exact determination. The determination gives high results when very large concentrations of bromate are encountered. Thus, titration of 0.1 N iodate to which sufficient solid potassium bromate had been added to make its concentration 1.8 N gave results about 1 per cent high.

In titrations with 0.01 N solutions the reaction between iodate and iodide was found to be inconveniently slow unless the ratio of biphthalate to iodate was increased considerably. By using 1 gram of biphthalate for 0.10 to 0.25 milliequivalent of iodate in 10 to 30 ml. of solution, fairly accurate results were obtained (Table III).

Acknowledgment

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Vacuum Desiccator for the Synthetic Organic Laboratory

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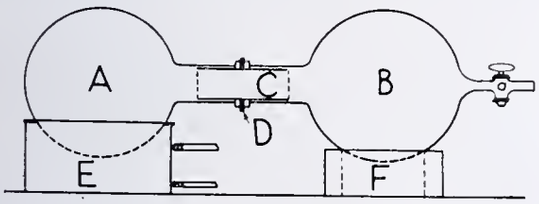
THE conventional form of a vacuum desiccator has limitations and disadvantages which render its use impracticable. In organic syntheses, whenever sharp drying of larger batches is required, the following simple adaptation of standard equipment has proved useful in this laboratory.



One of two similar round-bottomed flasks, B, is provided with a side arm for connection to the vacuum pump. (A glass stopcock in this place is desirable but not necessary. Its presence enables the flask to be used as a large separatory funnel.)

A not too loosely fitting glass sleeve, C, serves as a guide for the union between flasks A and B. The sleeve carries a rubber gasket, D, conveniently cut from an old inner tube. The gasket seals the vacuum, while the sleeve prevents the side-slipping of the contact surfaces. Without the guiding sleeve, the assembly is not safe. Special guiding clamps could be used, but they have no particular advantages in this case.

When in use, A contains the product, and B, the drying agent; E is a steam bath, and F, any convenient support. The apparatus, assembled at random from stock flasks of 5-liter capacity, will hold a vacuum of 2 mm. for 24 hours (with the aid of a high grade vacuum grease).

The following features make the "dumb-bell desiccator" particularly desirable for synthetic work: (1) Frequently one operation may be saved, since a round-bottomed flask in which the reaction or process of concentration was carried out may be attached to the drying bulb without the need of a transfer. (2) Within limits, the desiccator may be made as large or as small as desired. With reasonable precaution, flasks up to 24 liters may presumably be used. (The author has found it useful to coat evacuated vessels on the outside with a thin film of a soluble plastic. The shattering hazards seemed to be greatly reduced and experimentally induced implosions proceeded with not much more than a dull thud.) (3) While being dried the product may be readily heated on a steam bath and may be stirred by rotating the assembly. This also exposes a new surface of drying agent at each turn, thus hastening the process.

A comparative test with a highly hygroscopic sirup has shown that the dumb-bell desiccator works about twice as fast as the conventional model under comparable circumstances (such as equal amounts of desiccant). With gentle warming, the rate of drying was increased fourfold or better.

Communication No. 904 from the Kodak Research Laboratories.

Rapid Determination of Starch

An Index to Maturity in Starchy Vegetables

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IT IS well known that in certain vegetables such as peas, corn, and lima beans, the starch content of the seed tends to increase as the plant matures. This increase in starch is usually associated with a decrease in total sugar, tenderness, and general food quality of the vegetables.

Since the increase of starch is one of the major changes that occur during the maturing of these vegetables, it seemed logical that the estimation of starch should serve as an index of maturity and quality. This idea was supported by the fact that alcohol-insoluble solids, which give a rough measure of starch, correlate well with quality in green peas (*2*, *4*).

Common methods of starch analysis (*1*, *3*, *5*) usually involve drying the sample and grinding in a ball mill or other device for disintegrating the tissues. The material is then extracted with alcohol and suspended in water, the starch is brought into solution with acid, after which it is hydrolyzed with an enzyme or acid, the resulting product is clarified, and the sugar is determined by some conventional method. Some methods include an additional step for separating dextrins, either by different solubility in various concentrations of alcohol or by a precipitation of the starch with iodine, after which the starch is determined by hydrolysis or colorimetrically with iodine (*5*). Hassid and coworkers (*3*) combine the alcohol extraction and acid solubilization by adding a small amount of acid to the alcohol and refluxing.

The above procedures require a good deal of time-consuming manipulation and they increase considerably the chances of loss of starch. Most methods do not attempt to remove dextrins and other polysaccharides, which are determined along with starch upon hydrolysis. Pucher and Vickery (*5*) by a long procedure do remove most of the interfering substances.

A determination by any of the above methods will require at least a day and sometimes several days. If a large number of samples are run together a considerable amount of equipment is required. The method presented in this paper was developed mainly for speed without appreciable sacrifice in accuracy. Since manipulation is greatly reduced, errors from that source are minimized. The starch is estimated colorimetrically with iodine, using a red filter, which decreases considerably the error due to dextrins. Interfering glucosides and alkaloids are shown to be absent from the products examined. However, a rapid procedure for their removal is given, in case they are present in the sample to be analyzed. A single sample can be carried through the whole procedure, including preparation, in 20 to 30 minutes. Forty to 50 samples can be analyzed in a day.

Perchloric acid is unique, in that practically all its inorganic compounds are soluble in water. In order to ascertain whether this "solubilizing" effect might occur in organic combinations, various concentrations of perchloric acid were mixed with raw potato starch. It was found that after a certain normality was reached, which was well below the concentration of hydrochloric acid used for the same purpose, the starch granules lost their form and passed into solution in 3 to 4 minutes.

This acid "solubilization" has now been applied to vegetable material disintegrated in a Waring Blendor. As a result, a rapid procedure has been devised for the extraction of starch, which can then be estimated by the starch-iodine colorimetric method as suggested by Pucher and Vickery (*5*).

Reagents and Equipment

Photoelectric colorimeter. For rapid work the colorimeter should have a test tube adaptor and at least a dozen matched test tubes. A red filter is necessary for maximum sensitivity.

Disintegrator, a Waring Blendor or any similar instrument.

Perchloric acid, 72 per cent reagent grade solution; acetic acid, 2 *N* solution; sodium hydroxide, 6 *N* solution; potassium iodide, 10 per cent solution; and potassium iodate, 0.01 *N* solution.

Procedure

A 100-gram sample of the fresh, frozen, or canned vegetable is placed in the disintegrator cup with an equal weight of water, and the instrument is allowed to run at the high-speed position for 3 to 4 minutes. Two grams of the ground sample are weighed directly into a 30- or 50-ml. beaker on a torsion balance sensitive to 0.01 gram, 2 ml. of water are added, and then exactly 2.7 ml. of 72 per cent perchloric acid are slowly added with thorough stirring, so that there will not be momentary high concentrations of the acid in any portion of the sample. The mixture is allowed to stand with occasional stirring for about 10 minutes.

After standing, the mixture is made up to 25 or 50 ml. with distilled water, depending on the starch content, and then poured into a suitable test tube to settle. A 1-ml. aliquot of the supernatant liquid is pipetted into a 100-ml. beaker and 6 ml. of water are added. A drop of phenolphthalein is added and the solution is brought to a pink color with a few drops of 6 *N* sodium hydroxide. Now 2 *N* acetic acid is added until the pink color disappears, 2.5 ml. are then added in excess, 0.5 ml. of 10 per cent potassium iodide and 5 ml. of 0.01 *N* potassium iodate are accurately added, and the solution is allowed to stand at least 5 minutes. If the solution is relatively pale bluish-green, it should now be made up to 25 ml., and if it is dark bluish-green, it should be made up to 50 ml. The color is estimated in a photoelectric colorimeter using a red filter having a transmission range from 640 to 700 millimicrons. The colorimeter should be set at zero absorption or the readings corrected, with

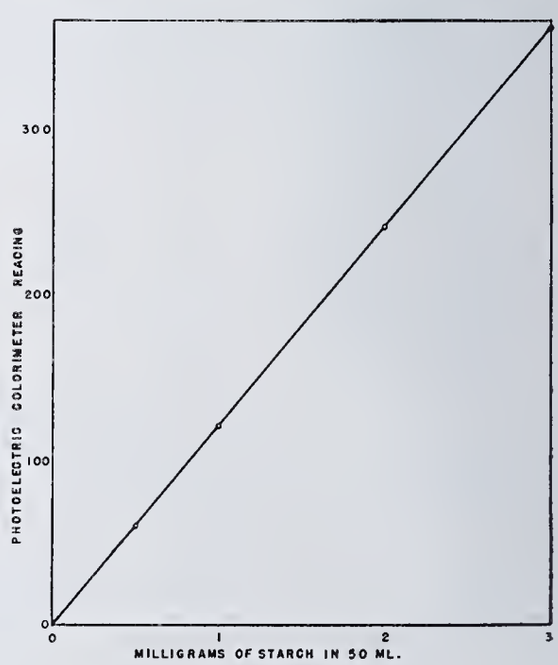

FIGURE 1. ABSORPTION OF LIGHT BY STARCH-IODINE SOLUTIONS, RED FILTER

Table I. Effect of Perchloric Acid Concentration on Solution and Hydrolysis of Potato Starch

Treatment	Weight of Starch	Molality of Acid	Starch Recovery
Min.	*Mg.*		%
10	35	12.0	11.0
10	35	8.0	16.2
10	35	6.0	89.8
10	35	4.8	100.0
10	35	3.4	87.5
10	35	2.7	67.8
15	35	12.0	4.1
15	35	8.0	12.4
15	35	6.0	80.0
15	35	4.8	100.0
15	35	4.0	100.0
15	35	3.4	94.5

a blank containing all of the reagents. If the filtrate used in developing the starch-iodine color is turbid, an extra blank correction should be made if precise results are desired. This can be done by discharging the blue color with a few drops of 0.1 *N* sodium thiosulfate and comparing the turbid solution against water.

Standardization

The percentage of starch is calculated from a curve prepared from the colorimeter readings of a known range of starch concentration. For solution depths of about 1.25 cm. (0.5 inch), the best range of starch concentrations is from 0 to 3 mg. per 50 ml. Figure 1 shows a typical standardization curve with the Klett-Summerson photoelectric colorimeter.

Soluble starch cannot be used for standardization. If absolute results are desired, starch prepared from raw, unblanched material similar to that which is to be analyzed should be used. Equal weights of starch from different products, such as potatoes and peas, do not give the same amount of color with iodine; therefore one type of starch cannot be used as a standard for all products. Perhaps if factors were established between the various starches, a single standard such as potato starch would suffice. If a series of analyses is to be carried out on a given product and only relative results are desired, potato starch, which is easily prepared, could be used to prepare a standard curve.

To obtain starch for standardization, disintegrate raw, unblanched material in a Waring Blendor type of disintegrator with an equal weight of water. Separate the fibrous material by washing the ground pulp through a 100- to 200-mesh screen, place the material that passed through the screen in a large beaker or pan, and stir with a large volume of water. Allow the starch to settle and decant off the water. Repeat this process until the starch is free of extraneous material. Now wash the starch with alcohol and ether and dry in an oven at 70° to 80° C. for 30 minutes. This should give a starch that is reasonably pure. Analyses can be made to establish its purity if such accuracy is desired.

Effect of Perchloric Acid Concentration on Solution and Hydrolysis of Starch

It was stated above that a certain concentration of perchloric acid had to be reached before the starch granules went into solution. To study this further and to ascertain the hydrolytic effect of the acid, samples of potato starch were treated with known concentrations of acid for two different periods. The data in Table I indicate that a concentration near 4 molal is necessary to bring the starch into solution and that this range can extend to at least 4.8 molal without hydrolysis of the starch when held at room temperature for 15 minutes. A similar experiment carried out on corn as shown in Table II placed the lower limit slightly higher, probably because of the buffering effect of the sample. In a third experiment on lima beans (Table III), little hydrolytic effect was shown even after 30 minutes.

Effect of Filtration on Solubilized Starch

A Whatman No. 1 filter paper can be used to filter the sample after it has been solubilized and made up to volume. Curiously enough, a Whatman No. 2 paper, which is a good deal heavier, removes about 20 per cent of the starch from the solution. For this reason the method was devised so as to involve no filtration and thus eliminate any chance of absorption of starch by filter paper. Turbid solutions which had settled for only a few minutes were sampled and compared with some of the same solution which had been centrifuged. Starch results by the two procedures were identical.

Stability of Starch-Iodine Color

If the sample is immediately made up to volume after the preparation of the starch-iodine color, a slow upward drift in colorimeter reading will be observed. However, if the sample is allowed to stand in the concentrated state for 5 minutes, this drift occurs to only a very limited extent. Twelve samples prepared by the latter procedure had an average upward drift of 1 division, equivalent to 0.008 mg. of starch in 40 minutes.

Table II. Influence of Perchloric Acid Concentration on Extraction of Starch from Corn

Sample No.	Molality of Acid	Starch Found in Sample
		%
73	4.8	5.20
	2.4	2.52
74	4.8	4.03
	4.0	3.41
	3.4	3.30
30	6.0	4.25
	4.8	4.83
31	6.0	2.83
	4.8	3.46
37	6.0	2.58
	4.8	3.20
45	6.0	3.13
	4.8	3.83

Table III. Influence of Time in 4.8 Molal Perchloric Acid on Per Cent of Starch Found in Lima Beans

Sample No.	Time in 4.8 *M* $HClO_4$	Starch Found in Sample
	Min.	%
66	5	7.34
66	10	7.25
66	15	7.17
66	30	7.15

Effect of Alcohol Extraction

Most of the procedures that have been proposed for starch involve drying the starch-containing material, grinding in a ball mill, and then extracting for several hours with alcohol. This serves to remove sugars which would interfere in a hydrolysis procedure for starch. According to Pucher and Vickery (*5*), it also removes certain compounds which give a blue color similar to that produced by starch with iodine. Since the method developed did not involve the determination of sugar produced by the hydrolysis of starch, the removal of sugars was of no concern. However, it is necessary to remove any compounds other than starch which would give a blue color with iodine.

To check this point, 20-gram samples of the disintegrated material from several different varieties of frozen corn, lima beans, and peas were each mixed with 200 ml. of alcohol and reground in the disintegrator for several minutes. A 25-ml. aliquot of this mixture was then filtered through a Whatman No. 1 paper in a Gooch crucible and washed several times

Table IV. Influence of Preliminary Alcohol Extraction on the Determination of Starch

Sample No.	Product	Starch	Starch after Alcohol Extraction
		%	%
86	Corn	2.43	2.45
87	Corn	1.45	1.46
36	Corn	3.86	3.91
30	Corn	4.83	4.86
31	Corn	3.46	3.52
37	Corn	3.20	3.28
45	Corn	3.83	3.91
51	Lima beans	9.35	9.30
882	Lima beans	12.12	11.85
877	Lima beans	12.42	12.52
874	Lima beans	11.91	12.00
3	Soybeans	2.43	2.50
57	Peas	7.90	7.62
10	Peas	9.30	9.10
36	Peas	7.92	7.94
5	Peas	7.80	7.58
59	Peas	10.90	10.50
65	Peas	9.35	9.13
27	Peas	6.65	6.50

with alcohol. That the extraction was complete was indicated by the pure white residue left on the filter paper. This residue was then suspended in 4 ml. of water and starch was determined by the procedure given above. Table IV shows that there were no interfering compounds in the products studied.

Influence of Dextrin

The method herein described does not attempt to separate dextrins from starch. It is not necessary in most cases and to do so would greatly lengthen the procedure. There was no indication of the presence of dextrins in the peas, soybeans, or lima beans used for the experiments. However, in corn an occasional sample had a slightly brownish color when iodine was added. To determine the influence of the red color of dextrins in the colorimeter reading using a red filter, a sample containing 125 mg. of bacteriological dextrin was analyzed. This sample gave an apparent starch content of 22 mg. or between one fifth and one sixth as much absorption of light

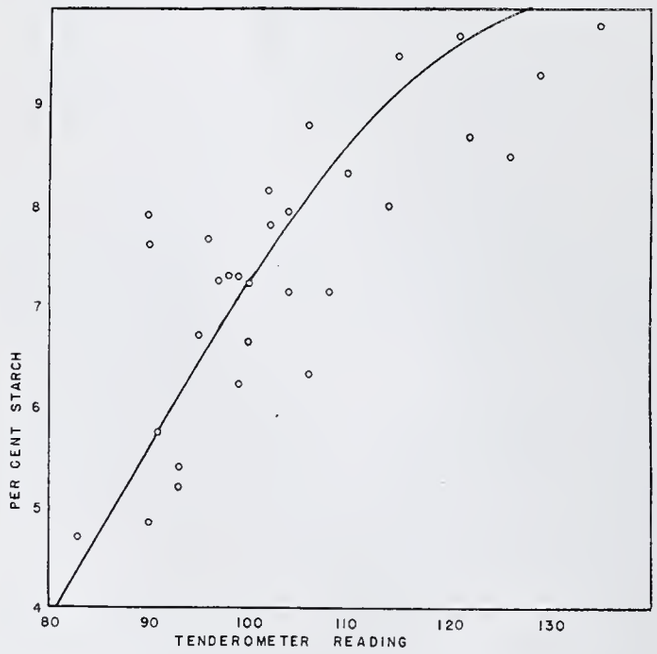

Figure 2. Relationship of Per Cent Starch to Tenderometer Reading in Raw Peas

Curve drawn by inspection

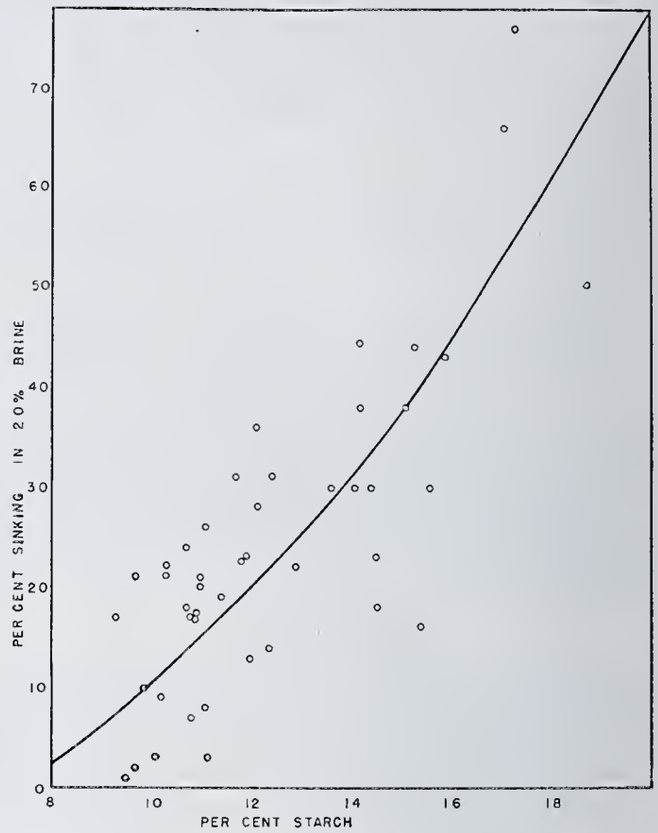

Figure 3. Brine Flotation of Baby Lima Beans *vs.* Per Cent Starch

Curve drawn by inspection

as would the same amount of starch. This means that the error due to dextrins is much less than in a hydrolysis procedure for starch where the dextrins are not removed.

Recovery Experiments

A known quantity of potato starch was added to samples of lima beans and soybeans whose starch contents had been previously determined. Table V indicates that the starch added can be determined within less than 3 per cent, which is a good recovery for most starch methods.

Table V. Recovery of Added Potato Starch

Product	Starch Added	Starch Found	Recovery
	Mg.	*Mg.*	%
Soybeans	0	25.6	
	49.3	74.2	98.6
Lima beans	0	109.6	
	49.3	158.0	98.2
	0	69.5	
	49.3	117.5	97.4

Discussion

Since the vegetables are to be analyzed for a constituent which is not in solution, great care should be taken to keep the disintegrated material well mixed as it is being weighed out. Care should also be taken not to add an excess of perchloric acid to the sample, since a few tenths of a milliliter of acid will cause a considerable increase in the final molality.

Perchloric acid develops some heat of solution. The rise in temperature brought about when the acid is added to extract the starch facilitates its solution. Cold 4.8 molal perchloric acid, however, will dissolve starch in 15 to 30 minutes. If the time period suggested in this paper is adhered to, 72 per cent acid must be used in order readily to get a sufficient rise in temperature to dissolve the starch in 5 to 10 minutes.

Complete extraction of the starch is indicated by recovery experiments, the ability to duplicate results closely, and the check of results on samples reground and extracted with alcohol with those obtained directly determined without extraction. Other evidence is that a reextraction of the residue on the filter paper after the extraction of starch gave a negligible starch content. A starch determination on material left on a 40-mesh screen after grinding the sample in the disintegrator and washing through the screen also gave a negligible result.

Further evidence of the reliability of the method has been obtained in its application. A group of raw pea samples were graded for quality with a tenderometer and then analyzed for starch. Figure 2 shows the relationship between these two factors.

Fifty samples of frozen lima beans were graded for quality organoleptically and by brine flotation. Starch analyses on these samples correlated very well with both the organoleptic and brine flotation grading. The relationship between proportion of sinkers in 20 per cent brine and percentage of starch in lima beans is shown as an example in Figure 3.

Rapidity of the Method

Starch in a single sample can be determined in 20 to 30 minutes if the reagents have been previously prepared. Forty to 50 samples can be analyzed for starch in an 8-hour day if a routine is established.

Perchloric Acid Hazards

There is no danger whatsoever from the 72 per cent perchloric acid itself, other than the usual hazards of strong acids. The cold diluted acid is a very poor oxidizing agent, as is evidenced by the fact that it will not release iodine from iodide ion. Hot perchloric acid, however, forms unstable compounds with organic material and for this reason the treated samples should never be heated. Samples have been allowed to stand in the laboratory at room temperature and to dry on filter paper for several days without accident.

Summary

A very rapid and reasonably accurate method for the determination of starch in certain vegetables has been developed. It includes grinding the fresh sample in a Waring Blendor-type disintegrator, extracting the starch with 4.0 to 4.8 molal perchloric acid, and estimating by photoelectric colorimeter the dissolved starch indicated by the blue color produced with iodine. Alcohol extraction of the products studied was found to be unnecessary. The use of a red filter in the colorimeter considerably reduces the error produced by dextrins when present.

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Separation of Carotenes from Xanthophylls

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THE methods which have been most generally used for the separation of xanthophylls from carotenes in provitamin A analysis suffer from certain disadvantages. The separation by extraction of petroleum ether solutions with 85 to 90 per cent methanol and the separation on various adsorption columns are time-consuming. The obvious advantages of the chromatographic method for routine work are offset by the difficulties in obtaining adsorbents of uniform performance, and by the fact that there always appears to be some loss of provitamin A material on the columns.

It was known that compounds containing conjugated double bond systems such as occur in the azulenes (*6*) react with 85 per cent orthophosphoric acid to form blue substances which are extracted from petroleum ether by the acid. It was thought that this reaction might be applied to carotene separations.

Petroleum ether solutions of the mixed pigments of canned pineapple, which were obtained by saponification of the solid material with 30 per cent potassium hydroxide in methanol for 12 hours at 3° C. and extraction with petroleum ether (60 to 70°), were treated with phosphoric acid; 25-ml. portions of the petroleum ether solution, which does not need to be dried, were shaken with 4 to 5 ml. of 85 per cent phosphoric acid in a 25-ml. glass-stoppered graduate. The acid layer became blue-green in color. The absorption curve for the pigment remaining in the petroleum ether solution was essentially the same as those obtained with carotene solutions which had been purified by the methanol separation and by passage through magnesia and calcium phosphate columns. Figure 1 shows a group of such curves compared with that of pure β-carotene (S. M. A. β-carotene).

The xanthophyll fraction obtained from a sample of these mixed pigments by the methanol separation was transferred into petroleum ether and this solution was treated with 85 per cent phosphoric acid. All the pigment was removed from the petroleum ether phase and converted into blue-green substances in the acid layer. On the other hand, carotene extracts, purified both by the methanol separation and by passage through magnesia or calcium phosphate columns, gave no apparent reaction when treated in the same manner with 85 per cent phosphoric acid. The absorption curves of such extracts were not appreciably altered by the phosphoric acid treatment (Figure 2). The deviation of the curve of a sample of phosphoric acid–treated β-carotene from that of the untreated carotene is similar to that obtained with extracts of natural materials. This change in the absorption of the pigments is undoubtedly due to isomerization of the type first observed by Gillam and El Ridi (*3*, *4*) and extensively studied by Zechmeister and his co-workers (*5*, *7*, *8*, *9*), and by Beadle and Zscheile (*2*).

Using this shortened method, results were obtained on canned pineapple which were in satisfactory agreement with those of biological assays (Table I). In this case the results can be expressed as units of vitamin A, since chromatographic adsorption on magnesia showed the pigment to be homogenous, and the absorption curve checked that of β-carotene which had been treated with phosphoric acid.

A comparison was made of the A. O. A. C. method (*1*) and the phosphoric acid method, and since carotene has a slight solubility in 90 per cent methanol the results are as might be expected (Table II). In each case the phosphoric acid method gave results which were slightly higher than those of the A. O. A. C. method.

Table I. Comparison of Phosphoric Acid Method and Biological Assay for Vitamin A Activity of Five Brands of Canned Pineapple

(International units per 100 grams)

Brand	H_3PO_4 Method	Rat Assay U. S. P.
A	135	145
B	106	125
C	129	143
D	134	138
E	135	147

Table II. Comparison of Phosphoric Acid and A. O. A. C. Methods

Sample	H_3PO_4 Method $\gamma/100$ g.	A. O. A. C. Method $\gamma/100$ g.
1	75.6	66.0
2	111.5	99.5
3	103.9	97.3
4	66.0	58.1
5	99.5	90.0
6	97.3	87.6
	Av. 92.3	83.1

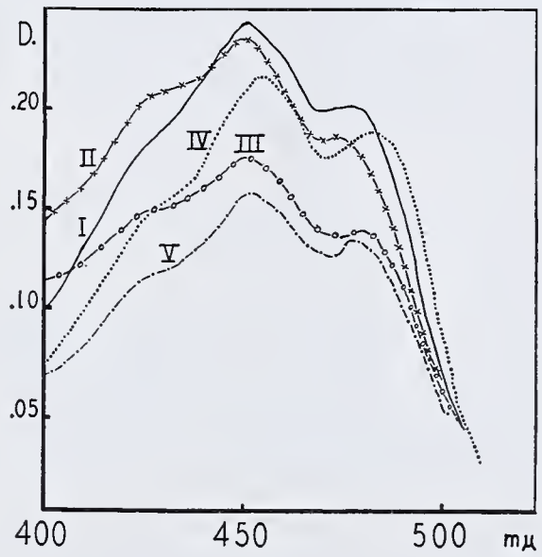

Figure 1. Comparison of Absorption Curves of β-Carotene Obtained by Various Separation Methods and of Pure β-Carotene

I. Adsorbed on Micron Brand magnesia
II. Separated with phosphoric acid reagent
III. Partition between petroleum ether and 90 per cent methanol
IV. Pure β-carotene
V. Filtered through dicalcium phosphate column

As a further check on the method, samples of mixed poultry feeds were saponified and extracted by the A. O. A. C. procedure. These extracts were treated with 85 per cent phosphoric acid and the absorption curves of the remaining pigments in the petroleum ether solution were measured. The curves were the same as those obtained for extracts separated by the use of 90 per cent methanol, and the concentrations of carotene calculated from the absorptions at 450 mμ were in agreement (Table III).

Summary

A new method which has been applied to the purification of carotene extracts in the determination of carotenes in foods, etc., makes use of the reaction between xanthophylls and 85 per cent orthophosphoric acid to separate the latter from carotene. Results agreed satisfactorily with the biological assays and also with the A. O. A. C. method. It is more rapid than the A. O. A. C. method and probably more accurate for analysis of material of low or average carotene content. This is due to the loss of carotene in the A. O. A. C. method occasioned by the residual solubility of carotene in 90 per cent methanol.

When the spectrophotometer is used it is preferable to make the measurements in the range of 440 to 450 mμ, since the absorption values of the extracts do not deviate greatly from those of β-carotene. As in all analytical methods, this method must be used with discretion, as, for example, phosphoric acid will not remove lycopene. It will not separate α- and β-carotene, and in such cases the method would yield the crude carotene content.

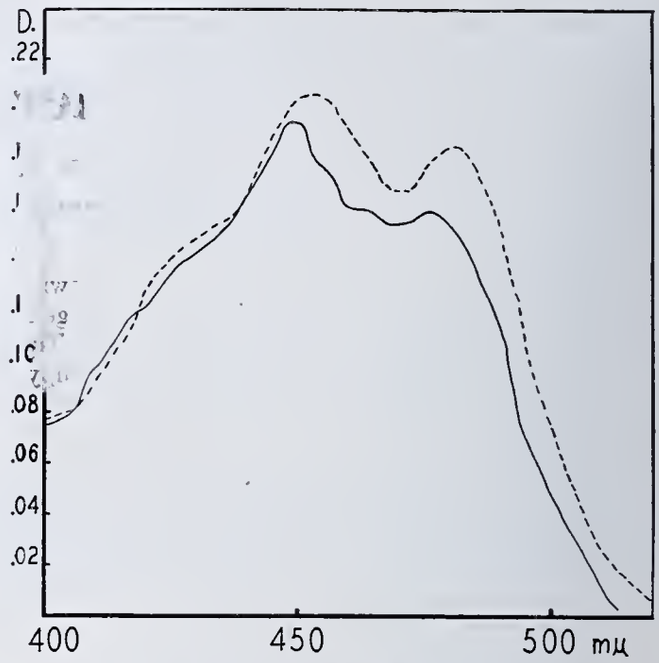

Figure 2. Absorption Curve of β-Carotene before and after Treatment with Phosphoric Acid

- - - Pure β-carotene
——— Pure β-carotene after shaking with phosphoric acid

Table III. Comparison of Phosphoric Acid and A. O. A. C. Methods on Mixed Poultry Feeds

Sample	H_3PO_4 Method γ/g.	A. O. A. C. Method γ/g.
1	13.3	11.0
2	15.3	12.4
3	13.8	11.5

Acknowledgment

The authors wish to acknowledge with thanks the Pineapple Producers Cooperative Association's financial support for the research program of which this investigation is a part.

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Chromogenic Reagent for Vitamin C Det minations

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RECENT studies in this laboratory have sho that ferridipyridyl sulfate may be used as a rea t for spectrophotometric determination of vitamin C. e reagent cannot be prepared directly by reacting ridyl with ferric sulfate, but a yellow-brown solution of the complex compound may be obtained by oxidizing ferrodipyridyl sulfate with ceric sulfate. Ferridipyridyl ion is fairly stable, and on reduction with vitamin C forms the extremely stable pink or deep red ferrodipyridyl ion. With ferridipyridyl in excess, the ferrodipyridyl formed is proportional to the amount of vitamin C. Beer's law is followed over a wide range, permitting accurate spectrophotometric determination of the vitamin.

The reagent oxidizes ascorbic acid (molecular weight 176.12) in two stages, corresponding to equivalent weights of 88.06 and 44.03, at widely different reaction velocities. It may thus be possible to determine vitamin C in the presence of other reducing substances, by suitable control of temperature, time, and concentration of the reagent. This possibility is to be investigated in an extension of the present work in which the new method is being compared with other methods for ascorbic acid in analyzing a series of increasingly complex systems.

Preparation of Reagents

Sulfur Dioxide Solution. Pass washed tank sulfur dioxide into a weighed portion of distilled water, reweigh, and dilute to obtain a 1 per cent solution.

Ceric Sulfate Solution. Add 28 ml. of 36 *N* sulfuric acid to 500 ml. of water. Add 40 grams of commercial ceric sulfate, stir until dissolved, filter after standing, and dilute to 1000 ml.

Aqua Ammonia. Bubble commercial anhydrous ammonia into distilled water until a 4 *N* to 6 *N* solution is obtained. Ordinary aqua ammonia is not suitable even when distilled, as it contains reducing impurities. Commercial synthetic anhydrous ammonia produced by the National Ammonia Co. of Philadelphia was found satisfactory.

Ferrodipyridyl Solution. Dissolve 1.76 grams of ferrous ammonium sulfate hexahydrate (Mohr's salt) in a little water and add 10 ml. of 1 per cent sulfur dioxide solution. Make to about 800 ml., add 2.50 grams of α,α'-dipyridyl (2,2′-bipyridine) and stir until this has dissolved. Heat to boiling (or allow to stand overnight) to complete the reaction, cool, and make to 1000 ml. The clear red ferrodipyridyl solution is very stable, and may be used as a stock solution for preparing the ferridipyridyl reagent in smaller portions.

Ferridipyridyl Reagent. Heat 200 ml. of ferrodipyridyl solution to 80° C. and titrate with ceric sulfate solution until the color changes from red to yellow; about 11 ml. of 0.08 *N* ceric sulfate are required. The end point is sharp but slow. Back-titrate with 1 per cent sulfur dioxide solution to restore a pink color.

With the aid of a pH meter adjust the solution to a pH of 3.6 by addition of aqua ammonia, avoiding even a temporary excess, which may cause ferric hydroxide to precipitate. The solution darkens as it is neutralized. Finally make to 400 ml. and if a precipitate forms allow it to settle out overnight and use the clear supernatant solution as the reagent. If necessary, add sulfur dioxide from time to time to maintain a small amount of ferrodipyridyl in the reagent. The effect of the ferrodipyridyl will be eliminated by use of a suitable blank as a reference solution in the transmittance measurements, whereas if it is absent an error may result. A check on this adjustment is provided by the blanks, which should be faint pink in color rather than yellow. The blanks prepared as described below are usually stable and each one may be used for all transmittance measurements made over a period of 2 or 3 days.

Acetate Buffer. Make 60.0 ml. of glacial acetic acid and 10.00 grams of recrystallized and suitably dried sodium acetate trihydrate to 1 liter, and adjust to a pH of 3.6 by further small additions of acid or salt.

Analytical Procedure

The following procedure is general, and may easily be adapted to meet particular requirements. The essential accuracy is between 0.5 and 2 per cent, but much larger errors may result from sampling difficulties, oxidation of the vitamin, and the presence of interfering substances.

The solution used in the transmittance measurements must be clear. Clarification may be effected by filtration through Pyrex or platinum filter crucibles, by centrifuging, or sometimes simply by allowing suspended or colloidal material to settle out. This may be done either before or after color formation; once the color has been fully developed it remains constant for days, in most cases.

Spectrophotometric Procedure. Add a small piece of Congo red paper to an aliquot portion of the analytical solution containing not more than 0.40 mg. of ascorbic acid. The volume of the aliquot should be such as to permit addition of reagents without exceeding the specified final volume, and should preferably be small, since dilution decreases the velocity of the analytical reaction.

Add aqua ammonia until the color of the indicator paper is the same as that of a similar piece in a buffer of pH 3.6. The pH is not critical, but should probably not be less than 2.5 or more than 4.0.

Next add 10 ml. of acetate buffer and 20 ml. of ferridipyridyl reagent and eventually (see below) make the solution to a volume of exactly 100 ml. The four critical amounts above may be halved or quartered without changing the transmittance-concentration curve, as a matter of convenience or economy: α,α'-dipyridyl is somewhat expensive. Moreover, the ferridipyridyl reagent addition specified represents a fivefold excess over the maximum ascorbic acid concentration measurable with 1.308-cm. cuvettes and might be reduced in some cases, the blank being changed accordingly.

Constant color may be attained to permit readings within 30 to 60 minutes by heating the solution between 70° and 80° C. for at least 25 minutes before cooling and dilution to final volume. Some systems—often those in which the reagent has been considerably diluted by the aliquot—become cloudy if heated too long or at too high a temperature, but usually give correct results after clarification. Alternatively, constant color may be developed by allowing the solution to stand at room temperature for 12 to 48 hours before dilution to final volume; this period of standing may conveniently be used to remove suspended or colloidal material. In either case the vitamin is oxidized to threonic acid and oxalic acid.

Transmittance readings made between 10 and 40 minutes after adding the chromogenic agent, with solutions kept at room temperature, indicate in many cases that the analytical reaction has proceeded almost exactly to the dehydroascorbic acid stage. This suggests that dehydroascorbic acid is formed rapidly and later oxidized more slowly to the other acids. Several interesting and perhaps useful implications of this observation are more or less evident.

When constant color has been attained, make duplicate transmittance readings with two successive portions of the solution at a wave length of 510 millimicrons. Use as a reference solution a blank containing 5 ml. of the buffer and 10 ml. of the reagent per 50 ml. Using the median transmittance found, read the result of the analysis from a previously prepared graph.

In the present work calibration data were obtained with a Coleman Model 10-S-30 spectrophotometer equipped with square cuvettes 1.308 cm. in depth, using freshly prepared standard solutions made at separate times over an extended period with

pure crystalline ascorbic acid from three different sources. An independent and most accurate calibration was derived by calculating the transmittance-concentration curve for ascorbic acid as ferrodipyridyl from an accurately established transmittance-concentration curve for iron as ferrodipyridyl, through the iron equivalent of ascorbic acid. All measurements were made at temperatures between 28° and 32° C.

The various calibrations in which the ascorbic acid was oxidized to threonic and oxalic acids are best represented by a straight line passing through the points (T = 100.0, C = 0.000) and (T = 10.0, C = 0.400) where T is the percentage transmittance of the solution relative to the blank plotted on a logarithmic scale, and C is the ascorbic acid concentration in milligrams per 100 ml., plotted on an arithmetical scale. The less well established line for oxidation to the dehydroascorbic acid stage passes through the point (T = 10.0, C = 0.80).

Colorimetric Procedure. Colorimetric measurements may be made by comparing analytical solutions obtained as described above with standards of similar composition prepared in the same manner and at the same time.

Applications of the Reagent

So far the new reagent has been used mainly in assaying commercial ascorbic acid, citrus fruit juices, and dried foods for ascorbic acid. It doubtless may also be used in determining other substances capable of reducing it quantitatively. However, both large and small concentrations of such common reducing agents as arsenious acid, formaldehyde, acetaldehyde, methyl alcohol, and formic acid do not reduce the reagent, either in hot or cold solution, and 0.4 mg. of oxalic acid dihydrate or 200 mg. of citric acid per 100 ml. have little or no effect on the reagent even in systems heated to 80° C. Sulfur dioxide reduces the reagent rapidly, but in small concentrations does not produce equivalent quantities of ferrodipyridyl when treated by the procedure used for ascorbic acid.

Photometric Determination of Reduced and Total Ascorbic Acid

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THE most widely used procedures for the determination of reduced ascorbic acid depend on its oxidation to dehydroascorbic acid. Rapid titration of an acid extract with a standardized solution of the sodium salt of 2,6-dichlorophenolindophenol yields accurate values for products rich in ascorbic acid and relatively free from interfering reducing substances. The photometric method (*2, 3, 15*) has made possible analyses of colored or turbid solutions even in the presence of other reducing substances. This procedure involves measurement in a photoelectric colorimeter of the progressive reduction of an excess of the dye.

Dehydroascorbic acid, the biologically active, reversibly oxidized form of the vitamin, must be reduced before it can be determined by any of the dye methods. Hydrogen sulfide is usually used. A microbiological reduction of dehydroascorbic acid employing a suspension of *Bacterium coli* has been described (*5*). However, this fails for some food materials.

In this report, a modification of the photoelectric colorimetric methods of Bessey (*2*) and Evelyn, Malloy, and Rosen (*3*) is presented. Fundamental studies of the applicability of the method to the determination of reduced and dehydroascorbic acids in a variety of biological materials were carried out. Evidence is submitted for the superiority of the photometric method over the visual, the necessity for including dehydroascorbic acid in the estimation of "vitamin C", and the proper procedures which should be observed in partitioning vitamin C between the reduced and dehydro forms.

Ascorbic Acid Determination, Biological Materials

Apparatus. A direct-reading photoelectric colorimeter with a 520-millimicron filter. (A null-point instrument cannot be used. The direct reading galvanometer must be critically damped and have a short period. The time required to swing from 0 to 100 and become stable should not be more than 3 seconds. An Evelyn photoelectric colorimeter, manufactured by the Rubicon Co., Philadelphia, Penna., has proved very satisfactory.)

Interchangeable test tubes for the colorimeter.

A pipet delivering exactly 5 cc. of an aqueous solution in less than 1 second. This is easily constructed from a 5-cc. transfer pipet as illustrated in Figure 1.

Reagents. The metaphosphoric acid and citrate solutions described by Bessey (*2, 16*).

A stock 2,6-dichlorophenolindophenol reagent containing approximately 10 mg. per 500 cc. The photometric density of 5 cc. of the dye plus 5 cc. of the citrate–metaphosphoric acid buffer at pH 3.5 is adjusted to read exactly 0.398 (transmission = 40.0 per cent) by dilution of the dye with water. (The galvanometer is first set at 0.0 for zero transmission, and 100.0 for a solution containing 5 cc. of the buffer at pH 3.5 and 5 cc. of dye, to which a crystal of ascorbic acid is added for complete decolorization.)

Procedure. All manipulations are conducted under an atmosphere of nitrogen, and all extractions and dilutions are made with solutions previously deaerated with a stream of nitrogen. This precaution is necessary if reduced ascorbic acid is to be determined but not if only the total vitamin C content is of interest.

A representative sample is dropped into an equal weight of 6 per cent metaphosphoric acid solution, and macerated mechanically. (A Waring Blendor has proved most satisfactory. One minute at high speed gives an excellent homogeneous mixture of most food substances.) For complete inhibition of the oxidase in some vegetables, it is necessary to add large segments of the sample directly to boiling 6 per cent metaphosphoric acid. (Boiling converts metaphosphoric acid in part to orthophosphoric acid, the latter solution having a lower pH. This, however, has little effect on the final pH, for subsequent dilutions are made with fresh metaphosphoric acid.) In such a medium the action of oxidative enzymes is inhibited. After boiling for 5 minutes, the contents are cooled, transferred to a Waring Blendor, and then macerated.

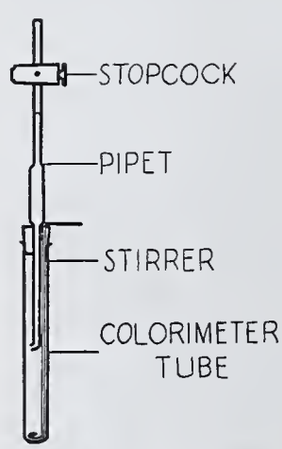

Figure 1. Pipet Used for Addition of Ascorbic Acid Extract to Dye Solution

By use of this technique as much as 150 grams of material may be taken for analysis. The desirability, and in many cases the necessity, of using such large quantities for proper sampling are obvious. This procedure is better than averaging a series of replicate tests on very small portions of the test material (*7*).

An aliquot of the uniform suspension is drawn off and diluted with 3 per cent metaphosphoric acid until the total solids content of the sample in suspension is 10 per cent or less. The samples are shaken mechanically for 15 minutes and then centrifuged. The extract is then buffered at pH 3.5 by adding 14 cc. of the citrate solution to 50 cc. of extract. Using graduated pipets, a rough visual titration is made to determine the volume of extract necessary to decolorize 5 cc. of the dye. Diluting this volume to 10 cc. with citrate–metaphosphoric acid buffer generally gives the optimal concentration for measurement in the photoelectric colorimeter. A testing range of 2 to 6 micrograms per cc. is considered optimal. Smaller concentrations cause insufficient reduction of the dye and hence give less precise results. Larger concentrations are to be avoided because the quantity of dye becomes a limiting factor and also because accurate galvanometer readings at the 5-second point are difficult when the transmission values are increasing rapidly.

Total ascorbic acid (reduced plus dehydro) is determined by a slight modification of Bessey's procedure (*2*). To 25 cc. of an extract buffered at pH 3.5, 6 drops of caprylic alcohol are added, and the solution is treated with a slow stream of hydrogen sulfide for 20 minutes. After 2 hours' standing in the closed system, the excess hydrogen sulfide is removed by streaming wet nitrogen through the solution for 2 hours.

For the photometric measurement the colorimeter tube is placed in the instrument and 5 cc. of the standard dye solution are introduced. The special pipet is filled with buffered extract, and with stirrer lowered, the assembly is placed on the tube as shown in Figure 1. The stopcock is opened, and 2 seconds later the assembly is lifted out of the tube. Galvanometer readings are taken exactly 5 and 10 seconds after the stopcock is opened. The absorption of the reaction mixture due to extraneous pigments and turbidities (the blank) is then obtained by adding a crystal of ascorbic acid to complete the decolorization of the dye.

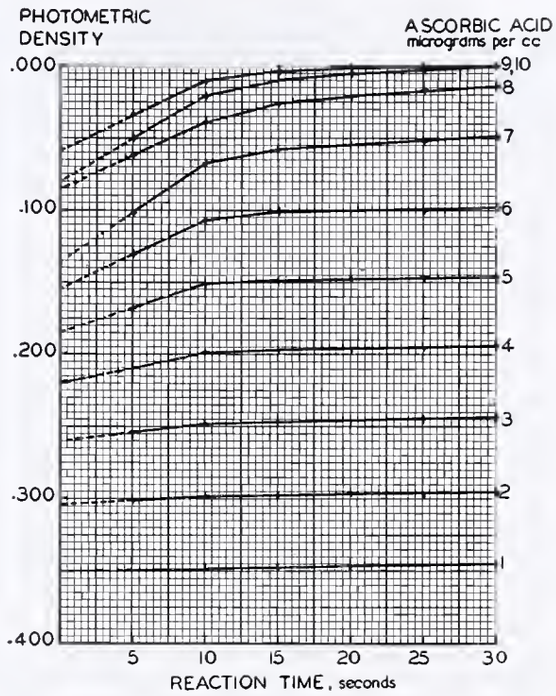

Figure 2. Progressive Decolorization of Dye with Increasing Concentrations of Ascorbic Acid

Reaction Rate between Dye and Ascorbic Acid. Figure 2 shows the rate of fading of the standard dye when it is mixed with solutions of ascorbic acid containing 1 to 10 micrograms per cc. Readings were also taken beyond 10 seconds for study of the reaction rate. In solutions containing less than 2 micrograms of ascorbic acid per cc., the reaction with the dye is almost instantaneous—i. e., complete in 5 seconds. Between 2 and 5 micrograms the reaction is incomplete at the end of 5 seconds but is complete at 10 seconds. For 6 micrograms fully 15 seconds are required for completion of the reaction, and concentrations from 7 to 8 micrograms fail to react completely within the 30-second observation period. Thus a concentration of ascorbic acid of 8 micrograms per cc., theoretically capable of completely reducing the dye, fails to do so after 30 seconds. Only when an excess of the vitamin is present, at least 9 micrograms per cc., is complete reduction of the dye observed during this period.

In the absence of the vitamin, the fading rate of the dye at pH 3.5 is approximately one tenth of a "per cent transmission unit" per 5 seconds. Accordingly, the reaction with the vitamin is considered complete when the rate of fading is equal to or less than this. In that case the extent of decolorization of the dye is observed to be proportional to the concentration of ascorbic acid in the extract (from 0 to 6 micrograms per cc. during the 30-second period).

In the equation

$$A = CD$$

where A is the concentration of ascorbic acid in the buffered extract, and D is the decrease in photometric density of the dye at the completion of the reaction, C is found to be 19.9 micrograms per cc. per unit of photometric density. Thus from a measurement of the decrease in the photometric density at any given instant, it is possible to calculate the extent of reaction at that time.

The reaction rate constant, k, for a second order reaction may be expressed by the equation

$$k = \frac{1}{(A - B)t} \log_{10} \frac{[B(A - X)]}{[A(B - X)]}$$

where A is the initial concentration of ascorbic acid in the reaction solution, B is the initial dye concentration also in the reaction solution, and X is the total decrease in concentration of each of the reacting components at any instant. From the data used in plotting Figure 2, the value of k may be calculated for each of the concentrations tested. Expressing the concentration of the dye, B, in terms of its ascorbic acid equivalent in micrograms per cc., A and X also in micrograms per cc., and t in seconds, the value for k is found to be 0.099 ± 0.015 at 25° C. and pH 3.5. This constant is confirmed by measurements with dye solutions of one third, one half, and twice the concentrations of the standard dye. (Because of the nature of the data the reaction constant is subject to much greater error than the ascorbic acid concentration calculated from the same data. The average deviation of the value for k was ±10 per cent, and k may be in error by 15 per cent; this value, however, is considered uniform for a reaction rate constant of the type measured here.)

The results of the study plotted in Figure 2 and supported by the reaction constant calculated therefrom constitute good evidence that the reaction between ascorbic acid and the dye is not instantaneous as has been claimed (*7*), and that the observed drift in galvanometer readings is due to incomplete reaction at the time of recording.

To avoid the complications arising from the fact that the reaction between dye and ascorbic acid is not instantaneous, two procedures were adopted by previous investigators for the measurement of the vitamin in biological extracts. One technique (*2*, *15*) involves taking readings at 15 and 30 seconds, neglecting the course of the reaction up to the first 15-second reading. However, results presented below indicate that many nonspecific reducing substances react to a considerable extent in 15 seconds, so that extrapolation of the 15- and 30-second readings obtained with extracts containing such substances yields erroneously high "ascorbic acid" values. A second alternative has been to take readings every 5 seconds (*3*) and to correct for the nonspecific reductants by extrapolation of the curve to zero time. This is a highly questionable procedure, for theoretically all curves should extrapolate to zero concentration of ascorbic acid at zero time because the reaction is not instantaneous.

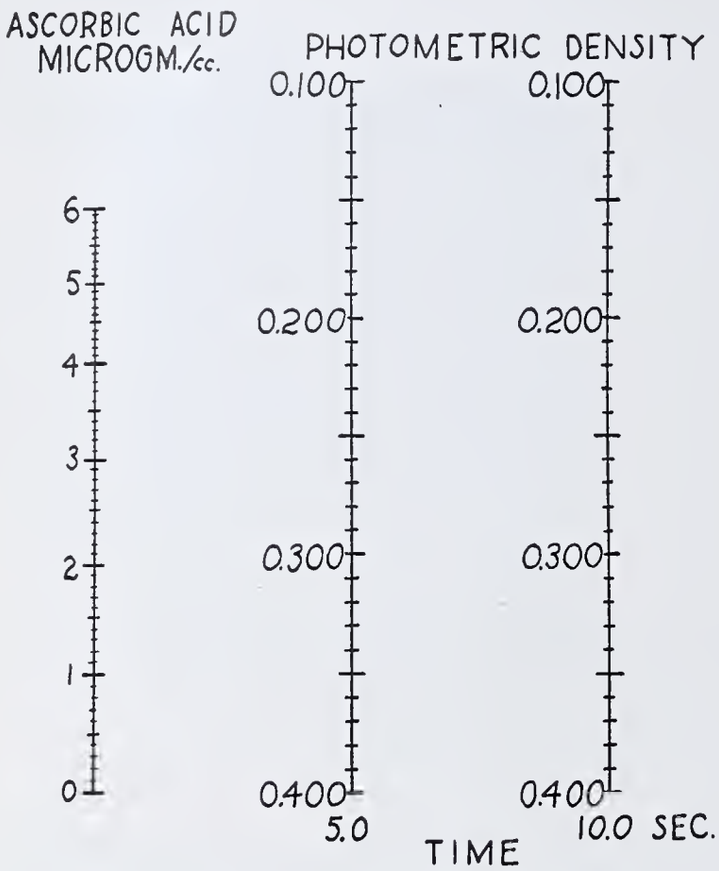

FIGURE 3. NOMOGRAM FOR ESTIMATION OF ASCORBIC ACID CONCENTRATION OF EXTRACT

Photometric density of blank is subtracted from those of test solution at 5 and 10 seconds. Straight-line extrapolation of corrected photometric densities gives vitamin concentration.

CALCULATION OF RESULTS. Anomogram (Figure 3) has been constructed to facilitate the estimation of the concentration of ascorbic acid in an extract. From the photometric densities of the test solutions at 5 and 10 seconds is subtracted that of the blank, and a straight-line extrapolation of the corrected photometric densities gives the vitamin concentration.

The nomogram was calibrated using the 0-, 5-, and 10-second values in Figure 2. This nomogram is merely illustrative and is not intended to be universally applicable. Each analyst should construct such a nomogram from data obtained in his own laboratory.

The extrapolation assumes that when two or more reducing substances are present their reaction curves (the residual photometric density of the dye plotted against time) are additive. If other reducing substances are present which react bimolecularly with the dye, as ascorbic acid does, the rate of disappearance of the dye is given by

$$\frac{-d\,[Dye]}{dt} = \sum K_i\,[R_i]\,[Dye]$$

where $[Dye]$ is the concentration of the dye at any instant; $[R_i]$ is the concentration of each reducing substance, and K_i is its bimolecular reaction constant. The rate of reaction in a complex mixture is thus a function of the concentrations of both the reducing substances and the dye. If a very large excess of dye were present, the reaction rate of each constituent in the mixture would be unaffected by the reaction of the other constituents; but with only a small excess of dye, the reaction of each reducing agent decreases the dye concentration sufficiently to affect significantly the reaction rates of the other constituents. Actual tests have been conducted on mixtures of ascorbic acid and other reducing substances, using the procedure described in this report and reported more fully below. These indicate that the negative error in the calculated ascorbic acid values arising from this cause is of the order of only a few per cent and is usually masked by the small positive error due to these interfering substances.

Comparison of Photometric and Titrimetric Determinations

The photometric method described above was compared with visual titration of 8 per cent acetic plus 2.5 per cent metaphosphoric acid (*8*) extracts of biological materials. The results are given in Table I. Agreement between the two methods is excellent for fresh orange juice, fresh tomato juice, and a pharmaceutical vitamin tablet. These products contain no appreciable amounts of nonspecific indophenol-reducing substances. For the other samples, however, the results differ considerably. Large discrepancies in the ascorbic acid values of the dehydrated products are noted. The vitamin capsule was manufactured 6 months before analysis and at the time of manufacture contained 35 mg. of ascorbic acid and 15 mg. of iron as ferrous sulfate. The results obtained with the capsule and the control solutions do not support the finding of Harris and Olliver (*7*) of no reduction of the dye by ferrous ion. In the photometric method extrapolation of the 5- and 10-second values reduces the interference of ferrous ion to one tenth of that evident in the visual titration. Woessner, Elvehjem, and Schuette (*21*) state "the ferrous ion reacts instantaneously with indophenol". However, in that study readings were taken only at 15 and 30 seconds. From the reaction curves for ferrous sulfate in Figure 4, it is apparent that a similar conclusion might be drawn from the authors' data if the 5- and 10-second readings were omitted. Ferrous

TABLE I. COMPARISON OF REDUCED ASCORBIC ACID VALUES AS DETERMINED PHOTOMETRICALLY AND TITRIMETRICALLY[a]

Material	Photometric	Titrimetric[b]
	Mg.	*Mg.*
Fresh orange juice, per 100 grams	49	48
Fresh tomato juice, per 100 grams	17	17
Vitamin tablet, per tablet	17	18
Canned tomato soup, per 100 grams (after 1 + 1 dilution)	2.0	3.5
Experimental dehydrated tomato soup, per 100 grams		
Fresh sample	26	30
After storage[c]		
No. 1	9	24
No. 2	7	24
No. 3	10	23
No. 4	8	22
Vitamin capsule containing $FeSO_4$, per capsule	21	45
$FeSO_4$ solution (10 mg. as Fe^{++}), per sample	1.5	15[d]
Ascorbic acid:$FeSO_4$ solution (10 mg.:10 mg. as Fe^{++}), per sample	11	24.5

[a] Using 2,6-dichlorophenolindophenol dye.
[b] Solvent = 8 per cent acetic acid, 2.5 per cent metaphosphoric acid.
[c] One week at 37° C. at 90 per cent humidity.
[d] This figure was also obtained using 5 per cent metaphosphoric acid as solvent.

TABLE II. REDUCED ASCORBIC ACID IN EXPERIMENTAL DEHYDRATED TOMATO SOUPS BY PHOTOMETRIC METHOD[a]

Sample	Initial Test	Incubated 1 Week, 37° C., 90% Humidity	Incubated 2 Weeks, 37° C., 90% Humidity
	Mg. per 100 grams		
1	26.2	8.7	1.2
2	28.4	7.1	2.1
3	21.3	9.8	0.3
4	27.2	7.7	1.2

[a] These samples had been judged by visual titration to be stable during the periods of storage, the ascorbic acid values approximating 30 mg. per 100 grams. However, organoleptic and colorimetric tests supported the conclusions drawn from the photometric values. Discrepancies in the figures obtained by the two assay procedures were not so great when the tests were conducted on subsequent products of improved stability in vitamin content and palatability.

iron is oxidized by atmospheric oxygen much more readily than ascorbic acid in the dilute solution used for photometric measurement. Therefore, in order to demonstrate the interference of ferrous iron, it is essential to exclude air from all solutions.

Visual titration with 2,6-dichlorophenolindophenol is frequently used to follow the fate of ascorbic acid in foods during processing and storage. The assumption is made that the concentration of interfering reducing substances is small, and is constant in the consecutive analyses. In the early stages of the development of a dehydrated tomato soup, visual titrations were used for control purposes for estimating reduced ascorbic acid. The results indicated the vitamin to be stable during the holding tests. When the photometric method was employed, very rapid destruction of the vitamin was observed. Typical values are listed in Table II. Apparently, other dye-reducing substances were formed during the storage period, and these were estimated as ascorbic acid by the titrimetric method. The same conclusion was reached in studies conducted by others (*12A*, *19A*) on dehydrated potatoes.

Importance of Including Dehydroascorbic Acid in Determining Vitamin C Content

Total ascorbic acid of the dehydrated soups was also determined by the photometric method. The results in Table III indicate that failure to determine dehydroascorbic acid causes the apparent loss of the vitamin to be much greater than that which actually occurred. The reaction curves for these samples have been plotted in Figure 5, after calculations to eliminate the effects of the ascorbic acid present. This allows graphic presentation of the progressive formation of other dye-reducing substances on storage. These compounds react slowly with the dye to give falsely high visual titrations. This interference is even greater after the hydrogen sulfide treatment.

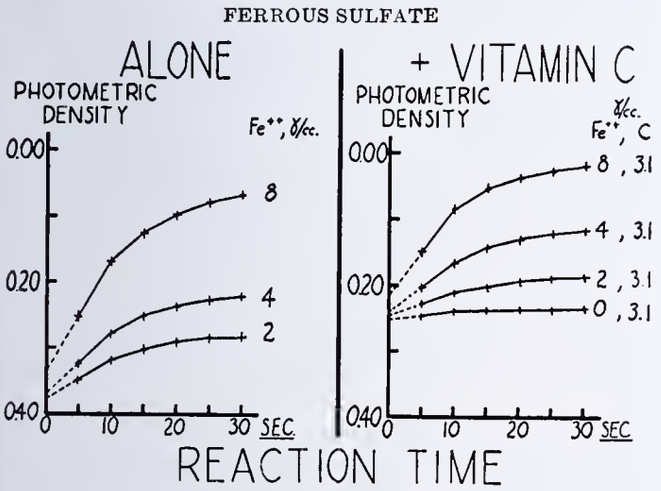

FIGURE 4. REACTION RATE CURVES FOR DYE AND FERROUS ION

With and without added ascorbic acid. Initial photometric density of dye is 0.398.

In Table III ascorbic acid analyses of a number of other foods by the photometric method are also presented. In the canned grapefruit juice, canned pineapple juice, and vitamin tablet, very little dehydroascorbic acid was found. The same was true of the tomato juice and orange juice when fresh, but after processing, appreciable amounts of the oxidized form of the vitamin appeared. After 2 weeks' storage at 37° C., 85 per cent of the ascorbic acid in the two dehydrated tomato soups and 26 per cent of that in the concentrated orange juice were in the biologically active, oxidized form.

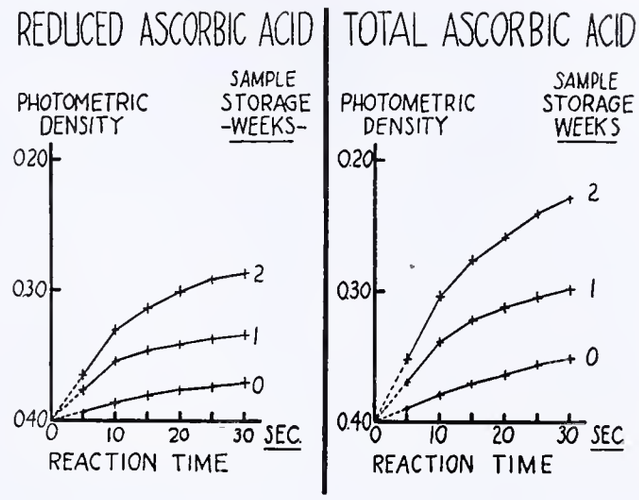

FIGURE 5. DEVELOPMENT OF DYE-REDUCING SUBSTANCES IN DEHYDRATED TOMATO SOUP DURING HOLDING TESTS

Initial photometric density of dye is 0.398.

Factors Operating to Convert Reduced to Dehydroascorbic Acid

Dehydroascorbic acid may be present in foods at the time of analysis, as shown in Table III, or it may arise in the course of analysis from oxidation of reduced ascorbic acid. A fundamental precaution in the analysis, if a reliable value for reduced ascorbic acid is to be obtained, is the prevention of atmospheric oxidation which is accelerated by oxidases and copper, especially with increasing temperature. Preparations of composite samples and of extracts are sometimes lengthy procedures, so that considerable oxidation of reduced ascorbic acid may occur if precautions are not taken.

In the analysis of a composite of a daily diet, the constituents were mixed with solid carbon dioxide, and the mixture was ground several times to produce a homogeneous semisolid mass. The manipulations required 2 hours, but at all times the temperature was maintained at less than 0° C. Photometric analysis for

TABLE III. REDUCED, DEHYDRO, AND TOTAL ASCORBIC ACID IN BIOLOGICAL MATERIALS DETERMINED BY THE PHOTOMETRIC METHOD

Material	Ascorbic Acid Reduced	Dehydro	Total
	Mg.	*Mg.*	*Mg.*
Canned grapefruit juice, per 100 cc.	40	1	41
Canned pineapple juice, per 100 cc.	35	0	35
Vitamin tablet, per tablet	17	1	18
Fresh tomato juice, per 100 grams	17	1	18
Fresh orange juice, per 100 grams	49	0	48
Raw, fresh string beans, per 100 grams	17	6	23
Orange sirup 2, per 100 grams	30	4	34
Stored 2 weeks, 42° C., per 100 grams	23	8	31
Experimental dehydrated tomato[a], per 100 grams	62	11	73
Experimental dehydrated tomato soup[b] (No. 1), per 100 grams	26	6	32
Stored 1 week, 37° C.	9	6	15
Stored 2 weeks, 37° C.	1	6	7
Experimental dehydrated, tomato soup[b] (No. 2), per 100 grams	27	5	32
Stored 1 week, 37° C.	8	6	14
Stored 2 weeks, 37° C.	1	6	7
Dehydrated potato, per 100 grams	0	0	0
Dehydrated banana, per 100 grams	2.5	0.8	3.3
Potato chips[c], per 100 grams	11	5	16
Pasteurized milk, per quart	0	3.6	3.6
Evaporated milk, per quart	0	0	0
Milk powder, per 100 grams	2.3	0	2.1
Composite diet, freshly prepared at less than 0° C. under CO_2, per 2710 calories	0	115	115

[a] Represents 12-fold concentration of tomato solids.
[b] Represents 5-fold concentration of tomato solids.
[c] In preparation of potato chips a 3.5-fold concentration of solids occurred. Thus, actual loss of ascorbic acid in making this product is calculated to be approximately 80 per cent.

Table IV. Influence of Temperature in the Determination of Reduced and Total Ascorbic Acid in Dehydrated Tomatoes[a]

(Values expressed in mg. per 100 grams of sample)

Ascorbic Acid	Initial	1 Hour	3 Hours	5 Hours
		At 0° C.		
Reduced	54	54	52	51
Dehydro	9	9	13	14
Total	63	63	65	65
		At Room Temperature		
Reduced	54	46	40	38
Dehydro	9	12	22	24
Total	63	58	62	62

[a] This product, representing a 12-fold concentration of tomato, was free from enzymes, but contained approximately 50 p. p. m. of copper. Acid extracts were allowed to stand for periods indicated.

Table V. Influence of Atmospheric Oxygen in the Determination of Reduced and Total Ascorbic Acid in Dehydrated Tomato Soups

Sample No.	Experiment	Ascorbic Acid Reduced	Dehydro	Total
		Mg. per 100 grams		
A-1	Extracting buffer saturated with air; analysis under air	27	16	43
A-2	Extracting buffer saturated with nitrogen; analysis under air	32	12	44
A-3[a]	Extracting buffer saturated with nitrogen; analysis under nitrogen	36	7	43
B-1	Same as A-1	23	11	34
B-2	Same as A-2	27	8	35
B-3[a]	Same as A-3	31	3	34

[a] Tests involving addition of 3 mg. of ascorbic acid to 10 grams of these samples gave recovery values of 92 and 98 per cent, respectively, as reduced ascorbic acid.

reduced ascorbic acid showed none to be present. However, after the hydrogen sulfide treatment, 115 mg. of total ascorbic acid were found. Calculation of the ascorbic acid content from published data on the component foods indicated this finding to approximate the expected value. The ascorbic acid was supplied mainly by fresh, uncooked fruits and vegetables; these included 150 cc. of orange and 75 cc. of tomato juice. From the nature of the foods in the diet, it was obvious that the dehydro was formed from reduced ascorbic acid (through the action of ascorbic acid oxidases) in the course of preparing the sample, despite the presence of carbon dioxide and maintenance of the low temperature. The dehydroascorbic acid apparently was stable under these conditions.

In an accurate measurement of reduced ascorbic acid, it is desirable to complete the analysis as soon as possible after extraction is effected. If delay is unavoidable, especially when a large series of analyses is being conducted, immersion of the samples in an ice bath inhibits oxidative changes. In Table IV values are given for reduced and total ascorbic acid in an acid extract (buffered at pH 3.5) of dehydrated tomatoes which was allowed to stand in air at room temperature and at 0° C. for various periods. Progressive decrease of reduced ascorbic acid was observed in the extract at room temperature with little change at 0° C. However, the values for total ascorbic acid were not affected by the preliminary oxidation. (These measurements were made immediately after removal of the excess hydrogen sulfide by nitrogen aeration.) The material analyzed was free from enzymes but contained an appreciable amount of copper (*13*).

The influence of atmospheric oxygen upon the oxidation of reduced ascorbic acid at room temperature is shown in Table V. In the first solution the extracting buffer was saturated with air and the analysis conducted in air. In the second the extracting buffer was saturated with nitrogen and the analysis conducted again in air; and in the third the extracting buffer was saturated with nitrogen and the analysis conducted under nitrogen. A progressive increase in reduced ascorbic acid was noted with the elimination of oxygen from the atmosphere. It is significant that here again the figures for total ascorbic acid were not affected by the partial oxidation.

In the analysis for reduced ascorbic acid in cooked and uncooked vegetables, the figures for the cooked products are frequently higher than those for the uncooked. This has been attributed by some (*17*) to the presence of bound ascorbic acid, which is released on cooking. Others (*12*) have ascribed the observation to the presence of active enzymes in the raw samples, which are inactivated during cooking. In Table VI are presented the results of some analyses on string beans, a product rich in ascorbic acid oxidase. Sample 1 was prepared in a Waring Blendor by a 1-minute, high-speed maceration of the raw beans with water under carbon dioxide. Sample 2 was homogenized with 6 per cent metaphosphoric acid instead of water. Sample 3 was blanched for 5 minutes in boiling water, cooled, and then macerated in the cooking water. The results of this study, summarized in Table VI, emphasize the precautions which must be observed to prevent enzymic oxidation of the ascorbic acid. The thermal inactivation of the enzymes by boiling in 6 per cent metaphosphoric acid appears to be the preferred method. Use of 5 per cent sulfuric–2 per cent metaphosphoric acid as the extracting solvent (*14*), with no preliminary blanching of the beans, yielded essentially the same results for the reduced and dehydroascorbic acid content. Extraction with the stronger acid is not so desirable, since subsequent pH adjustment cannot be made so readily as in the procedure recommended in the present report. It is significant that the figures for total ascorbic acid in all samples were the same. These observations support Harris and Olliver's criticism (*7*) of the bound ascorbic acid theory (*17*), since the total ascorbic acid content of the unblanched beans is not increased on cooking. The enzymic oxidation taking place in the uncooked product accounts for an apparent increase in "ascorbic acid" after cooking when reduced ascorbic acid alone is measured.

Applicability of Photometric Method to Urine Analysis

Dehydroascorbic acid is reported (*1*) to be fairly stable in pure solutions below pH 5. The stability of reduced and total ascorbic acid was investigated photometrically in a composite sample of fresh urine obtained from twelve subjects and stored under air. For comparison with the photometric method, the usual visual titrations for reduced ascorbic acid were also conducted. The results are presented in Table VII. The visual titration for reduced ascorbic acid on the freshly voided urine gave results more than 100 per cent greater than those yielded by the more specific photometric method. This confirms the findings of Roe (*19*) who used an independent analytical approach. Another series of tests with added crystalline ascorbic acid (100 mg. per liter) indicated good agreement between both methods, due to the fact that at the higher dilutions used in the test the concentrations of nonspecific reducing substances were considerably lower. (This is comparable to generally observed values for the urine of normal subjects following oral administration of a 300-mg. test dose.)

Table VI. Influence of Ascorbic Acid Oxidase in the Determination of Reduced and Total Ascorbic Acid in Raw String Beans

(Values expressed in mg. per 100 grams of beans)

Sample No.	Preparation of Sample[a]	Ascorbic Acid Reduced	Dehydro	Tota
1	Raw, macerated with equal weight of H_2O	4	19	23
2	Raw, macerated with equal weight of 6% HPO_3 solution	14	9	23
3	Boiled with H_2O 5 minutes, then macerated	16	7	23
4	Boiled with 6% HPO_3 solution 5 minutes, then macerated	17	6	23

[a] All manipulations were carried out under an atmosphere of carbon dioxide.

Table VII. Stability of Ascorbic Acid and Other Dye-Reducing Substances in Urine

(Values expressed in mg. per liter)

Preservative per Liter	Form	Ascorbic Acid before and after Storage: Initial Photometric	Initial Titrimetric	After 24 Hours Photometric	After 24 Hours Titrimetric	After 48 Hours[b] Photometric	After 48 Hours[b] Titrimetric	After 120 Hours Photometric	After 120 Hours Titrimetric
None	Reduced	8	19[a]	3	13	0	4	0	0
	Dehydro	9	..	3	..	1	..	4	..
	Total	17	..	6	..	1	..	4	..
30 cc. of 3.5 *N* H_2SO_4	Reduced	11	23	9	19	3	13	0	6
	Dehydro	5	..	4	..	4	..	6	..
	Total	16	..	13	..	7	..	6	..
10 grams of HPO_3	Reduced	8	20	4	13	2	13	0	6
	Dehydro	10	..	4	..	3	..	0	..
	Total	18	..	8	..	5	..	0	..
10 grams of HPO_3 and storage at 5° C.	Reduced	9	20	8	23	1	14	1	12
	Dehydro	9	..	8	..	11	..	9	..
	Total	18	..	16	..	12	..	10	..

[a] Comparisons should be made between only reduced ascorbic acid values in evaluating degree of nonspecificity of titrimetric method. Total ascorbic acid values, obtained by titrimetric method, are greater, but such analyses of urine are practically never made.

[b] Ascorbic acid values obtained after this period of storage lack precision because of lowered concentration of vitamin in presence of large amounts of interfering reducing substances in urine. This is particularly true of dehydroascorbic acid figures which are obtained by difference.

The ascorbic acid in the samples containing the larger quantities appeared to be appreciably more stable. This is in agreement with frequent observations (*4*) that the oxidative destruction of ascorbic acid is more nearly absolute than proportional to the concentration of the vitamin. Storage of the urine sample at 5° C. with added metaphosphoric acid was the most effective means for preservation. A consideration of the data compiled indicates that the nonspecific reducing substances in urine are also unstable but not to the same extent as ascorbic acid.

Extrapolation of Photometric Densities at Five and Ten Seconds

Linear extrapolation of the photometric density at 5 and 10 seconds gives ascorbic acid values considerably lower than extrapolation at 15 and 30 seconds. This may arise from greater specificity of the former method, or from a possible early retardation of the rate of reaction between the dye and ascorbic acid by other substances in biological extracts. If such a retardation were a reality, low recoveries of added ascorbic acid would be obtained by the 5- and 10-second extrapolation procedure. Accordingly, recovery tests were conducted on a variety of biological materials. The values shown in Table VIII are representative of the differences in ascorbic acid values to be expected in the photometric method using the two extrapolation procedures, and indicate that extrapolation of 5- and 10-second readings furnishes a more specific index of ascorbic acid content.

Compounds Interfering with the Photometric Method

The use of hydrogen sulfide for the determination of dehydroascorbic acid has been criticized (*6, 9, 10, 11, 14*). It has been asserted (*8, 14*) that nitrogen aeration fails to remove the last traces of hydrogen sulfide, which in turn could be responsible in part for increased "ascorbic acid" figures. Under the conditions of test described here, there is insufficient residual hydrogen sulfide to cause any photometrically measurable dye reduction even after only 20 minutes of nitrogen aeration of a pure buffered solution previously saturated with the gas, or of a similarly treated solution with added ascorbic acid. This is confirmatory of the experiments of Hamburger and Joslyn (*6*). Reference is made to Figure 6 giving the reaction curves for two solutions of hydrogen sulfide (not subjected to nitrogen aeration) which were able to decolorize the dye. By using the 5- and 10-second extrapolation this interference is reduced by more than 70 per cent.

It has further been suggested (*6, 9, 10, 14*) that the hydrogen sulfide is loosely combined in biological extracts or is responsible for the formation of nonvitamin dye-reducing substances. However, alleged interferences from these causes were based for the most part upon titration values without extrapolation to zero time.

In the present study tests were conducted with three of the compounds which have been reported (*20*) to react with hydrogen sulfide to yield indophenol-reducing substances. The reaction curves for these are also plotted in Figure 6. In these graphs, the "ascorbic acid" concentration is 0.0 microgram per cc. at a photometric density of 0.398, and 4.0 at a photometric density of 0.200. The solid curves represent reaction curves after hydrogen sulfide treatment (with subsequent removal of the gas). The values at 5 and 10 seconds are extrapolated to zero time by means of a straight line as on the nomogram. (The dotted curves obtained prior to hydrogen sulfide reduction indicate that these compounds cause no decolorization of the dye.) The results show that except for 2-methyl-1,4-naphthoquinone, the interferences following the hydrogen sulfide treatment are much greater in the visual titration (single value at the 15-second or 30-second point) than in the photometric method. In fact, pyruvic acid shows no interference, for its curve extrapolates linearly to a photo-

Table VIII. Justification for Extrapolation of Photometric Densities at 5 and 10 Seconds in Preference to 15 and 30 Seconds

Test Material	Ascorbic Acid from Extrapolation of Photometric Densities at 5 and 10 seconds (*a*)	15 and 30 seconds (*b*)	Value[a] for $\frac{(b)-(a)}{(a)} \times 100$	Recovery of Added[b] Ascorbic Acid from Extrapolated Photometric Densities at 5 and 10 Seconds
	Micrograms per cc. of extract			%
Dehydrated tomato soup A-3	2.93	3.80	30	104
Dehydrated tomato soup B-3	2.34	2.81	20	93
Dehydrated banana	1.63	2.61	60	93
Dehydrated potato	0	0	...	96
Pasteurized milk	0.91	1.15	26	105
Evaporated milk	0	0	...	90
Milk powder	1.07	1.39	30	103
Urine, composite of 12 subjects	1.81	2.15	20	94
Ferrous sulfate, 5 micrograms of Fe^{++} per cc.	0.74	2.25	204	97[c]
Orange, fresh	4.18	4.18	0	No recovery tests conducted on these samples
Tomato, fresh	5.62	5.94	6	
Urine	2.32	3.22	39	
Urine during vitamin C saturation test	2.97	3.14	6	
Pineapple pie	0.60	1.83	205	
Pumpkin pie	0.59	1.49	153	

[a] Per cent difference in ascorbic acid values obtained by two methods of extrapolation.

[b] Except where indicated, amount of ascorbic acid added for recovery experiment was approximately equal to amount believed to be originally present as determined from data in literature, or by previous analysis of same or similar samples. For recovery test, vitamin was added to original material and carried through entire analysis.

[c] For recovery test on this sample, 3.05 micrograms of ascorbic acid were added to each 1.00 cc. of test solution.

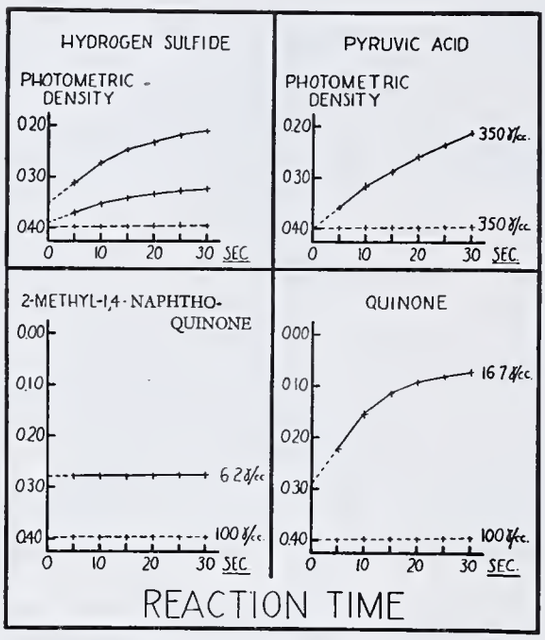

FIGURE 6. REACTION CURVES FOR SUBSTANCES ALLEGED TO INTERFERE IN DETERMINATION OF ASCORBIC ACID

Before (dotted lines) and after (solid lines) hydrogen sulfide treatment. Initial photometric density of dye is 0.398.

metric density of 0.398. For the others, the concentrations normally found in biological materials are not sufficient to interfere in the determination of total ascorbic acid. Thus, the objections to the use of the hydrogen sulfide reduction procedure in titrimetric or photometric methods previously published are minimized in the procedure described in the present report.

Discussion

In titrimetric methods in general, as the titration progresses the concentration of at least one of the reactants decreases. Thus, from kinetic theory the reaction rate must decrease. The behavior of the sulfhydryl compounds on dye titration reported by King (*11*) is entirely analogous to that noted in this laboratory with ferrous sulfate. Indeed, at the concentrations used in the photometric method, the reaction rate between ferrous sulfate and the dye becomes easily measurable (see Figure 4). As a consequence of this slower reaction, the interference of ferrous sulfate is reduced by 90 per cent in the photometric method (see Table I). Other interferences may be similarly minimized.

The results presented here impose serious limitations on the use of the visual titration. The method of "reversed titration" (*7*), titrating the dye with the extract, is inadvisable, for many of the interfering reducing substances added to the dye during the early part of the titration are given an ample period for reaction. The interference is thus much greater than in the normal titration where the dye is removed as it is added, chiefly by the more rapidly reacting ascorbic acid. The question of duration of the end point is controversial, for with a 5-second end point the ascorbic acid may not react completely, and with a 30-second end point, many interfering substances may react. In this laboratory, interfering reducing substances have been encountered in such a wide variety of samples that the photometric method has replaced visual titration. Only in the routine testing of pharmaceutical products shown by the photometric reaction curve to be free from interfering compounds is visual titration used.

The data presented in this paper on the occurrence of dehydroascorbic acid in biological materials and on the necessity for its determination are contrary to the conclusion of Harris and Olliver (*7*), who state, "The amount of vitamin C present in foodstuffs in the form of dehydroascorbic acid, even during aging and in stale foods, is generally so small as to be of little or no practical significance." However, their conclusion was based on analyses of a limited variety of foods by the "reversed titration" method, involving also an obviously faulty procedure for the conversion of reduced to dehydroascorbic acid, particularly with reference to pH adjustment (*14*).

Summary

Reduced and total ascorbic acid in biological materials have been determined by a modification of published photometric methods based on the rate of decolorization of the dye 2,6-dichlorophenolindophenol. A nomogram has been presented to facilitate the estimation of the ascorbic acid concentration from the residual photometric densities of the dye at the end of 5 and 10 seconds of reaction. The reduction of the dye by the vitamin was found to be a reaction of the second order; the rate constant was calculated for 25° C. and pH 3.5. The photometric procedure has a much greater degree of specificity than the visual titration, and allows determinations to be made on extracts containing small amounts of ascorbic acid, even in the presence of relatively large amounts of other substances which reduce the dye. Evidence has been obtained stressing the importance of determining dehydroascorbic acid, initially present in some materials and produced in others when proper analytical precautions are not taken. The photometric method is applicable to analyses of urine before and after the administration of test doses. Proof is presented of the greater specificity of the improved photometric method, as compared with the various titrimetric and photometric procedures.

Acknowledgment

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Determination of Ferrous Iron in Difficultly Soluble Materials

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THE determination of ferrous iron in materials containing both ferrous and ferric iron presents several difficulties when the material to be analyzed is difficultly soluble, as is the mineral chromite. Not only is it difficult to get the ore into solution, but the solvents oxidize the ferrous iron, usually without liberating another material which can be determined.

The method herein described indirectly determines ferrous iron by titrating the sulfur dioxide liberated when sulfuric acid oxidizes ferrous oxide.

Various methods have been proposed, but for one reason or another none has proved entirely satisfactory.

As early as 1860, Mitscherlich (*11*) determined ferrous oxide in chrome ore by decomposing the ore with medium strength sulfuric acid in a bomb tube at 250° to 290° C. The decomposition required about 8 hours, and precautions were taken to prevent oxidation by the air. Similar results were obtained by Jannasch and Vogtherrr (*8*), using hydrochloric acid–ammonium chloride solutions in a bomb tube. Not all chrome ores respond to this treatment, however.

Fusion with boric acid and treatment with a mixture of sulfur and hydrofluoric acid have also been tried (*7*). Decomposition with concentrated sulfuric acid has been found to be incomplete (*2, 15*), and treatment with acid sulfates such as potassium bisulfate oxidizes the chrome ore completely (*1, 6, 12*), as is the case also with Usatenko's phosphoric-sulfuric acid method (*18*). Treatment with oxidizing acids such as nitric acid is impracticable (*4, 10, 19*).

Alkaline fusions, using sodium carbonate or borax (*3, 5, 13, 14*), absorb oxygen from the air rapidly at the high fusion temperature required.

Schein (*16*) measures the increase in weight of the ferrous oxide on heating in oxygen, making allowance for the loss of water and carbon dioxide in the process. The unusually high calculating factor, 9, is an objection to this method. The author has determined the oxidizable materials in the ore by first finding the loss on ignition in an atmosphere of nitrogen and the subsequent gain when heated in oxygen. The results agree very closely with those obtained by the procedure described in this paper.

Methods recently proposed depend upon solution of the ore in phosphoric acid, the ferrous iron in the solution being then determined by titration with potassium permanganate. Konopicky and Caesar (*9*) dissolve 0.2 gram of powdered ore in 16 to 20 ml. of phosphoric acid of specific gravity 1.92 in a wide test tube. The test tube is heated on an air bath to 300° to 320° C. and carbon dioxide is passed through it during the reaction. The cooled solution is diluted with air-free water and titrated with permanganate to a gray color. In the author's experience this method gives results which are not only much too low but incapable of being duplicated. This is true because any sulfur trioxide present is reduced to sulfur dioxide which is lost, and at high temperatures phosphine may be lost. By this method duplicate results cannot be consistently obtained on magnetite of known ferrous oxide content.

Stevens (*17*) describes a method in which a 0.3-gram sample of ore is placed in a glass tube about 20 cm. long, 11 mm. in inside diameter, and 1 mm. in wall thickness. Five milliliters of 85 per cent phosphoric acid are added, the air in the tube is displaced by carbon dioxide, and the tube is sealed off by drawing it out in the form of a hook, at a point about twice the height of the phosphoric acid. The tube is then suspended about 1.25 cm. (0.5 inch) above the bottom of a metal crucible which is heated just strongly enough to keep the phosphoric acid gently boiling until the sample is dissolved. This may take anything up to 24 hours. After cooling, the tube is opened and the contents are washed into distilled water containing 10 ml. of sulfuric acid. This solution is then titrated with 0.05 *N* potassium permanganate to a gray color. (Incidentally, none of the authors mentioned seems to have made use of *o*-phenanthroline–ferrous sulfate complex as indicator in this titration.) This method has the same objectionable features as the Konopicky and Caesar method described above.

It has been found possible to obtain consistent results by this method, but they are again too low, and the correction to be applied, as determined by experiments with magnetite, is so large as to preclude use of the method for materials containing low percentages of ferrous oxide. Another disadvantage is the length of time required for a determination.

Stevens notes that the need for a correction factor is due to the reduction of some of the phosphoric acid and consequent oxidation of some of the ferrous oxide. The extent of this oxidation probably depends on the time of heating and on the amount of ferrous oxide present, and since these factors vary with different ores, a single correction factor cannot very well serve for all ores. Sulfur trioxide and other impurities in the phosphoric acid yield gaseous reducing reaction products which are partially lost. If the product of reduction is phosphorous acid, as assumed, the amount of the latter formed should be equivalent to the amount of ferrous oxide oxidized. Phosphorous acid, however, is not oxidized by permanganate solutions in the cold under these conditions, and thus cannot be determined by titration with permanganate.

In order to eliminate the large factor involved in the Stevens method, by estimating the phosphorous acid formed as well as the ferrous iron in solution, an iodometric method was tried. Theoretically, either ferrous or ferric iron may be determined iodometrically in a solution containing both, in accordance with the reversible equation

$$2Fe(OH)_2 + I_2 + 2H_2O \rightleftharpoons 2Fe(OH)_3 + 2HI$$

In strongly acid solution (hydrochloric acid) the reaction proceeds toward the left. In the presence of a small amount of sodium bicarbonate, which keeps the pH of the solution constant, the reaction proceeds toward the right, and should, therefore, constitute a method for the determination of ferrous iron. Tests were carried out using the methods, the phosphoric acid solution of the ore being neutralized by adding sodium hydroxide solution until nearly neutral and then adding solid sodium bicarbonate. An excess of standard iodine solution was then added, and the excess was titrated with sodium thiosulfate. The results obtained were still too low, and it was found that phosphoric acid could not be effectively neutralized with sodium bicarbonate.

Phosphorous acid decomposes on heating, phosphoric acid being formed and phosphine liberated in accordance with the equation

$$4H_3PO_3 = 3H_3PO_4 + PH_3$$

Assuming that acid solutions of phosphates behave in the same way, the reaction between ferrous oxide and phosphoric acid might take place in the following stages:

1. The ferrous oxide dissolves in the phosphoric acid, forming ferrous phosphate

$$3FeO + 2H_3PO_4 = Fe_3(PO_4)_2 + 3H_2O$$

2. The ferrous phosphate reacts with a further quantity of phosphoric acid, forming ferric phosphate, ferric phosphite, and phosphorous acid

$$8Fe_3(PO_4)_2 + 11H_3PO_4 = 15FePO_4 + 9FePO_3 + 3H_3PO_3 + 12H_2O$$

TABLE I. DETERMINATION OF FERROUS IRON

Weight Taken	Found by Operator A	Found by Operator B
Mg.	*Mg.*	*Mg.*
Estimation of Fe in Iron Wire		
25	25.4	26.7
50	50.3	51.1
75	74.8	75.8
100	99.3	101.4
125	123.9	125.7
150	149.9	149.9
Estimation of FeO in Cuban Chromite		
200	8.4	9.0
300	8.6	9.0
400	8.8	8.9
500	8.8	8.9
600	8.7	8.9
Estimation of FeO in Magnetite		
200	25.6	26.4
400	25.9	26.4
600	25.6	26.4
800	25.7	26.0

	Weight Taken	Found by Operator A	Found by Operator B
	Mg.	*Mg.*	*Mg.*
Determination of FeO in Chromites			
Cuban	500	8.2	8.2
	500	8.9	9.0
Transvaal	500	16.9	16.7
	500	16.8	16.7
Low-grade Philippine	500	8.3	8.5
	500	8.3	8.5
High-grade Philippine	500	10.8	11.2
	500	10.8	11.3
Rhodesian	500	10.4	10.7
	500	10.5	10.8
Turkish	500	8.9	8.6
	500	9.0	8.6
Blanks, Determination of FeO in C. P. Fe_2O_3			
	500	0.0	0.0
	500	0.0	0.0
	500	0.0	0.0
	500	0.0	0.0

3. The ferric phosphite–phosphorous acid mixture decomposes under the influence of heat, giving ferric phosphate and phosphine

$$3FePO_3 + H_3PO_3 = 3FePO_4 + PH_3$$

These equations may be combined and the net result of the reactions expressed as

$$8FeO + 9H_3PO_4 = 8FePO_4 + PH_3 + 12H_2O$$

Phosphine is oxidized by potassium dichromate, giving phosphoric acid

$$PH_3 + 2O_2 = H_3PO_4$$

It was decided to attempt the determination of ferrous oxide on these lines. The sample was heated strongly with phosphoric acid in a stream of carbon dioxide, and the gaseous products of the reaction were passed into standard 0.1 *N* potassium dichromate solution. From the amount of dichromate reduced, the ferrous oxide was calculated, using the last two equations. Any unchanged ferrous phosphate was determined by titrating the phosphoric acid solution with potassium permanganate.

At the time these experiments were started no pure phosphoric acid was available and C. P. phosphoric acid of specific gravity 1.57 was used. With this acid, the method was found to give excellent results with Cuban chrome ore containing about 9 per cent of ferrous oxide, but with a magnetite of known ferrous oxide content low results were obtained (Table I). Moreover, in the case of chrome ore the amount of residual ferrous iron in the phosphoric acid solution was found to be so small that the permanganate titration could be omitted, but with magnetite this titration amounted to 4 ml. or more, using a 0.5-gram sample. When the experiments were repeated later, using pure phosphoric acid, no reduction of the dichromate took place, but titration of the phosphoric acid solutions with permanganate gave results averaging 22.3 per cent ferrous oxide with a magnetite of 26.3 per cent ferrous oxide content, and 7.2 per cent ferrous oxide with a chrome ore containing 9.2 per cent ferrous oxide.

The reduced dichromate solutions obtained when C. P. phosphoric acid was used were analyzed for phosphates, and showed a phosphate content equivalent to only 0.26 per cent of ferrous oxide calculated on the phosphine theory. This amount of phosphoric acid could easily have been carried over mechanically into the dichromate. Furthermore, when chrome ore or magnetite was heated with either pure or C. P. phosphoric acid in a stream of carbon dioxide, and the gaseous reaction products were passed into mercuric chloride solution, a slowly forming white precipitate of calomel was obtained which contained no phosphorus. There was no trace of the light brown chloride-phosphide precipitate which forms in large quantities in the mercuric chloride when a small quantity of phosphorous acid is heated strongly with phosphoric acid under similar conditions.

It was, therefore, concluded that the reduction of the dichromate when C. P. phosphoric acid was used was caused by a gaseous product formed by the reaction of the ferrous oxide with some impurity in the acid rather than with the acid itself. Analysis of the C. P. phosphoric acid showed that, among other impurities, sulfates were present in considerable quantity. At high temperatures and in the presence of phosphoric acid the reaction between ferrous oxide and sulfuric acid may be represented by the equations

$$FeO + H_2SO_4 = FeSO_4 + H_2O$$

$$2FeSO_4 + 2H_2SO_4 = Fe_2(SO_4)_3 + SO_2 + 2H_2O$$

or simply

$$2FeO + 4H_2SO_4 = Fe_2(SO_4)_3 + SO_2 + 4H_2O$$

The sulfur dioxide passes into the dichromate and is oxidized to sulfuric acid. The amount of dichromate reduced, for the same amount of ferrous oxide, is the same whether the gas formed is phosphine or sulfur dioxide. Chrome ore was dissolved in C. P. phosphoric acid and the gases were passed into neutral dichromate solution. The sulfate content of the reduced dichromate solution was estimated gravimetrically as barium sulfate, and calculated to ferrous oxide. The results obtained were all high, owing to a small amount of sulfur trioxide carried over, but were in proportion to the results calculated from the titration of the reduced dichromate.

It was thus proved that the reduction of the dichromate was due to sulfur dioxide formed by the reduction of sulfuric acid by ferrous oxide, and that this reaction was quantitative.

The amount of sulfate in the C. P. phosphoric acid used was insufficient to oxidize all the ferrous oxide in a 0.5-gram sample of magnetite and this probably accounts for the low results obtained. A mixture of 1 part of sulfuric acid and 4 parts of phosphoric acid was found to give excellent results with all ores, including magnetite, and the resultant solution contained no ferrous iron. This mixture was, therefore, used in all subsequent experiments.

Chromium may also be determined in the phosphoric acid solution. The chromium is oxidized with ammonium persulfate in the presence of a silver salt, and the resulting chromate determined by titration.

Apparatus and Procedure

Reducing agents, such as ferrous iron, carbides, and sulfides, react at high temperatures (350° C.) and under low pressures with sulfuric acid in phosphoric acid to form sulfur dioxide, which is distilled into an excess of 0.1 *N* potassium dichromate. The excess potassium dichromate is determined and the reducing agent is calculated.

Chemicals Required for Ferrous Oxide Determination. Phosphoric-sulfuric acid mixture, 4 parts of 85 per cent phosphoric acid to 1 part of concentrated sulfuric acid; potassium dichromate, 0.1 *N* containing 50 ml. of sulfuric acid per liter; sodium thiosulfate, 0.1 *N;* potassium iodide, 40 per cent solution; and starch solution.

Apparatus. The apparatus used is shown in Figure 1. The sample and acid are contained in the 200-ml. round-bottomed reaction flask, *A*. A round-bottomed flask is necessary not only because the system is under a partial vacuum during the determination, but also because it is much more difficult to get chromium ores into solution in a flat-bottomed flask, the ore collecting around the bottom edge of the flask.

The reaction flask rests on the electrically heated air bath, *B*. The heating element is covered by an iron plate, and the heater supports an iron ring, which in turn supports a cover made of thick asbestos board. The cover has two holes for each flask, one of which is actually above the air bath, whereas the other is used to hold the flask while it is cooling after the reaction. The holes are tapered to fit the flask, and, using a 200-ml. flask and a a 1000-watt heater, should be 6.56 cm. (2.625 inches) in diameter at the bottom. The iron ring is made of such depth that there will be 2.5 cm. (1 inch) of space between the iron plate covering the heating element and the cover of the air bath. The temperature of the bath is controlled by a rheostat and ammeter, and is measured by a thermometer passing through a hole in the cover and suspended from a stout wire bent as shown.

The stopper of the reaction flask carries two tubes, one of which is connected with a supply of carbon dioxide contained in the large aspirator bottle, *C*. Prior to the determination the aspirator is completely filled with air-free water, which is then displaced by carbon dioxide from bottle *C*, and forced over into the other large bottle, *D*. Carbon dioxide of the highest purity should be used and it is recommended that the carbon dioxide be generated by adding cold freshly boiled dilute sulfuric acid to a strong solution of sodium bicarbonate which has been previously boiled and cooled. Cylinder carbon dioxide of the highest purity may be used, but it is not recommended because of the wide variation in the purity of different cylinders of gas. During the determination a slow stream of carbon dioxide is drawn from *C* by way of tube *E* through the whole apparatus. (Two tubes *E* are shown in the diagram, but only one is necessary.) *E* contains water and is of help in adjusting the rate of flow of the carbon dioxide.

The other tube leading from the reaction flask is connected with the absorption tubes, *F* and *G*, which contain the standard dichromate solution. These tubes are immersed in a vessel through which passes a stream of water for cooling purposes. The gaseous products of the reaction, together with carbon dioxide, bubble through the dichromate, which absorbs any reducing agent. Trap *H* is inserted as a precaution against the loss of dichromate by splashing, which may occur if too rapid a stream of carbon dioxide is passed.

The whole system is under a vacuum of some 680 mm. of mercury throughout the determination, and any possibility of the entry of air is precluded by the insertion of the Bunsen valve, *J*, in the line.

If the equipment is available, the rubber-stoppered reaction flask can be replaced by one having a ground-glass stopper with glass inlet and outlet tubes, and glass stopcocks can be used wherever metal pinchcocks are indicated in the drawing. The Bunsen valve can likewise be replaced by a glass float valve. Precautions, however, must be taken to avoid sticking of the ground-glass joints.

Preparation of Sample. Since all reducing agents, such as metallics, carbides, sulfides, etc., react with phosphoric acid, the preparation of the sample is important. Many chromites, after heat-treating, are magnetic and, therefore, removal of iron by a magnet is precluded.

The sample is crushed carefully in hardened steel, then ground in a porcelain ball mill with nonmetallic balls until all passes 100 B. S. mesh. A 10-gram sample is ground to pass through a 325-mesh Bureau of Standards screen in an agate or mullite mortar. Fine grinding is essential because of the time of solution.

Procedure. Twenty-five milliliters of the phosphoric-sulfuric acid mixture are placed in flask *A*, and 0.5 gram of the 325-mesh ore is introduced on top of the acid. When the sample has become wetted, the liquid is swirled in the flask until all small lumps have been broken up. Fifteen milliliters of 0.1 *N* potassium dichromate are placed in absorption tube *F* and 5 ml. in *G*, diluted with 10 ml. of water. Clamp *K* and stopcock *L* are then closed, and the apparatus is connected as shown in the diagram, *A*, resting in the cooling hole of the air bath for the time being. The vacuum pump is then started and *L* opened slowly, care being taken to avoid excessive splashing in the absorption tubes as the evacuation of the apparatus proceeds. When the stopcock is fully open and bubbling in the tubes has ceased, *K* is opened carefully and carbon dioxide is allowed to pass at the rate of about one bubble per second, as observed in *E*. *A* is then placed in the hole over the air bath, and is held in position by inverted U-shaped wire supports which stand in holes drilled

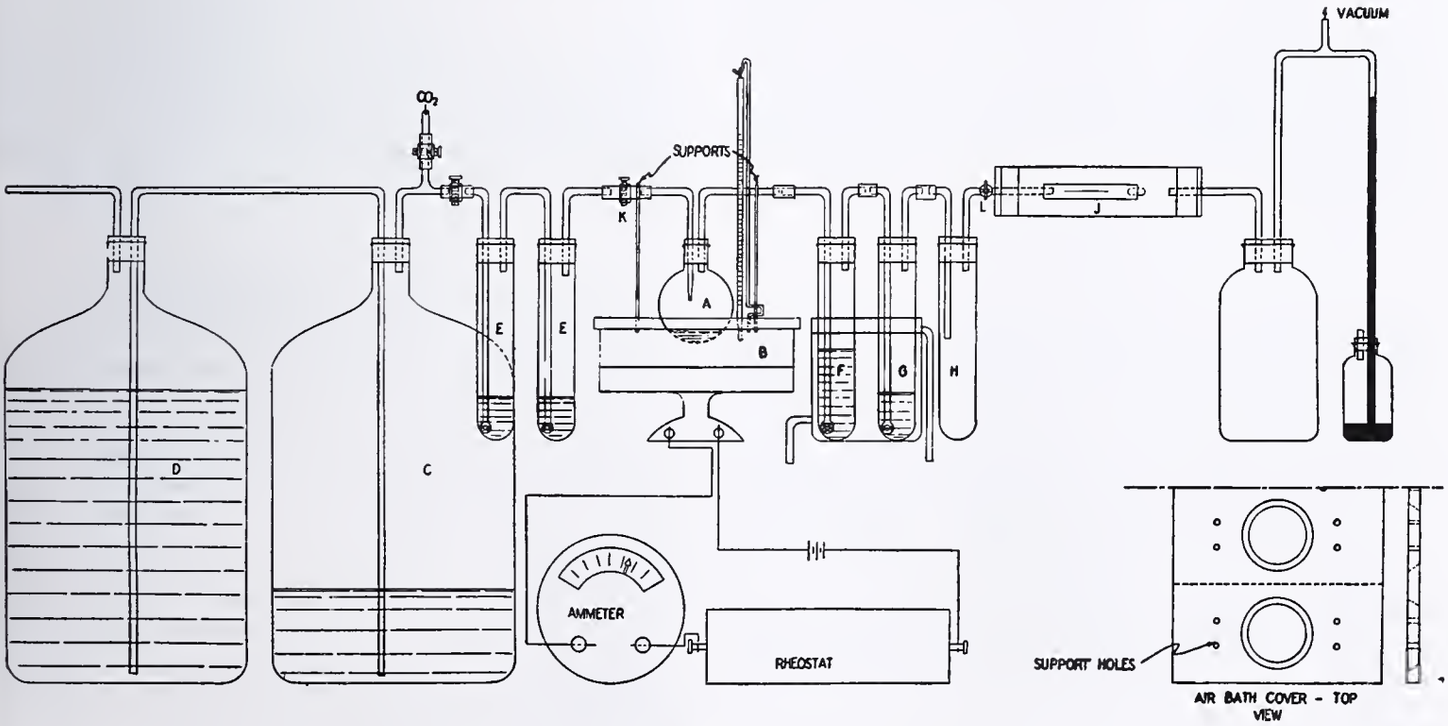

Figure 1. Apparatus for Determining Ferrous Iron

in the air bath cover. The bath is maintained at a temperature of 360° to 380° C.

Little or no solution of the ore takes place until all the water has evaporated from the acid mixture, after which the ore dissolves fairly rapidly. The time required for completion of the reaction varies from 35 to 90 minutes, depending on the ore. With some ores it is necessary to raise the temperature of the bath somewhat, but this should be avoided if possible, as the silica liberated from the glass of the flask coats the remaining particles of ore, rendering solution even more difficult. The reaction is complete at the point when bubbles cease to form in the acid mixture, but heating is continued for about 10 minutes after this point is reached.

The flask is then removed to the cooling hole of the air bath and the rate of flow of carbon dioxide is increased somewhat in order to avoid sucking back of the liquid in the absorption tubes and to ensure complete reaction between the sulfur dioxide and the potassium dichromate. After about 5 minutes *L* is closed and more carbon dioxide is admitted until the pressure has reached equilibrium throughout the system.

The absorption tubes are disconnected and the contents poured into a 500-ml. Erlenmeyer flask. The tubes and connections are carefully washed and the washings added to the main solution. Five milliliters of 1 to 1 sulfuric acid are added and the solution is diluted to about 250 ml. An excess of 0.05 *N* ferrous sulfate solution is added, using *o*-phenanthroline as an indicator. Not less than 10 ml. is added, and the excess is titrated, using 0.05 *N* potassium permanganate solution.

Alternate. Two milliliters of 40 per cent potassium iodide solution are added, and the liberated iodine is titrated against 0.1 *N* sodium thiosulfate to a green color having a faint yellow tinge. Starch solution is added and the titration completed, the end point being reached when the solution loses its blue color and becomes a pale apple green. This end point is sharp.

Since phosphoric acid varies considerably in impurities, it is necessary to determine the blank which must be deducted from each determination. The blank includes impurities in the acid and the amounts of standard solutions used to indicate the end point, and in no case should exceed 0.5 ml. If the solvent, consisting of 4 parts of phosphoric acid and 1 part of sulfuric acid, is fumed for 30 minutes at atmospheric pressure, the blank to be deducted from the determination approaches zero very closely. Boiling the solvent is considered good practice.

Calculation. The equation

$$2FeO + 4H_2SO_4 = Fe_2(SO_4)_3 + SO_2 + 4H_2O$$

shows that 2 moles of ferrous oxide liberate 1 mole of sulfur dioxide, which is equivalent to 0.5 mole of oxygen or 2 liters of 1 *N* potassium dichromate. Hence 1 liter of 0.1 *N* dichromate is equivalent to 0.1 mole of ferrous oxide in the determination, so that if *A* ml. is the combined volume of 0.1 *N* potassium dichromate used in the two absorption tubes, and *B* ml. of 0.1 *N* sodium thiosulfate is required to titrate the iodine liberated by the dichromate remaining after the reaction, $(A-B)$ ml. of potassium dichromate has been reduced. Then if *W* gram is the weight of the sample

$$\text{Percentage of FeO} = 0.7185 \times \frac{A - B}{W}$$

Limitations of Test. It is clear that the method will fail if the sample contains carbon, sulfides, metallics, or any reducing substance other than ferrous oxide, as these will also liberate sulfur dioxide from the sulfuric acid. (It is possible to remove reducing substances from chrome ore by ignition in air at 1000° F. without appreciably altering the ferrous iron content.) On the other hand, they may sometimes be used for the determination of these reducing substances if present alone. Experiments with pure iron were found to give accurate results by this method.

Determination of Chromium. The acid solution obtained during the determination of ferrous iron in a chromium ore may be used for the determination of chromium, eliminating the need for a sodium peroxide fusion.

Water is added to the flask while still hot. If the phosphoric acid solution is allowed to cool too much it becomes very thick and viscid, and is difficult to dissolve. The solution is washed into a 600-ml. beaker and diluted to 200 ml., 25 ml. of 1 to 1 sulfuric acid, 1 ml. of 1 per cent silver nitrate (catalyst), 50 ml. of 40 per cent ammonium persulfate, and at least 5 or 6 drops of strong potassium permanganate solution (2.5 per cent) are added, and the solution is boiled for 10 minutes. The presence of manganese is necessary to obtain complete oxidation of the chromium during this operation. The amount of manganese in most chrome ores is insufficient. Two milliliters of 1 to 1 hydrochloric acid are then added and the boiling is continued for a further 5 minutes, precipitating the silver and destroying any permanganate that may have been formed from manganese present in the ore.

The cooled solution is diluted to 350 ml. and 6 drops of *o*-phenanthroline indicator are added. Standard ferrous sulfate (approximately 0.2 *N*) is added from a buret, with stirring, until the liquid turns red, and then about 10 ml. more. The excess of ferrous sulfate is found by titration with 0.1 *N* potassium permanganate until the solution assumes a clear green color.

Alternate. The solution is cooled, 2 ml. of 40 per cent potassium iodide are added, and the iodine liberated is titrated against 0.1 *N* sodium thiosulfate. The thiosulfate titration is directly proportional to the chromium content of the ore.

The equation

$$2Cr_2O_3 + 3O_2 = 4CrO_3$$

shows that 12 liters of 1 *N* permanganate are equivalent to 304 grams of chromium trioxide, so that 1 ml. of 0.1 *N* potassium permanganate is equivalent to 0.002533 gram of chromium trioxide. If the amount of ferrous sulfate added is equivalent to *A* ml. of 0.1 *N* potassium permanganate and the final titration is *B* ml. of 0.1 *N* potassium permanganate, the chromium trioxide in the sample is equivalent to $(A - B)$ ml. of 0.1 *N* potassium permanganate. Hence, if the weight of the sample of chrome ore is *W* gram

$$\text{Percentage of } Cr_2O_3 = 0.2533 \times \frac{(A - B)}{W}$$

Summary

The method outlined is not perfect, but it gives the most accurate results of any method which has come to the author's attention. It is satisfactory for control. Boiling the solvent, 4 parts of phosphoric acid and 1 part of sulfuric acid, for 30 minutes decreases the blank to an exceedingly low value.

Acknowledgment

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Baumé–Dextrose Equivalent–Dry Substance Tables for Corn Sirup and Corn Sugar

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Tables covering the Baumé–dextrose equivalent–dry substance relationship for corn sirup and corn sugar sirup are presented. These are based on extensive research on a method (previously presented) for determination of dry substance. Dry substance for a given Baumé increases with increasing dextrose equivalent. Commercial Baumé is defined as Bé. = Bé. 140°/60° F.+1.00°. The tables have been accepted by the Corn Industries Research Foundation for use by its member companies, all refiners of the corn wet-milling industry.

APPROXIMATELY 1,500,000,000 pounds of corn sirup and 125,000,000 pounds of crude corn sugar are produced annually in the United States. The confectioners' industry is the largest consumer of corn sirup, although substantial quantities are used by the baking, canning, preserving, mixed table sirups, and ice cream industries. Crude sugars in either billet or chipped form—No. "70" and No. "80"—are used in the manufacture of rayon, leather, caramel color, and tobacco.

Corn sirup is the thick, viscous, substantially colorless sirup obtained from the incomplete hydrolysis of starch. It is sold on the basis of Baumé and dextrose equivalent and in the trade is often referred to as corn sirup unmixed or, more simply, C. S. U. The Baumé (commercial) will run from 42° to 47°, the bulk being 43°. Dextrose equivalent (D. E.) is defined within the industry as the percentage of reducing sugars calculated as dextrose, expressed on a dry substance basis. Under this general classification, commercial sirup falls roughly into four groups: brewers' body sirup, 25 to 35 D. E.; standard confectioners' sirups, 40 to 45 D. E.; extra sweet sirups, 50 to 57 D. E.; and dual conversion sirup (acid plus enzyme), 60 to 70 D. E. The names for the first three groups are general, in that there is considerable interchange in the use of these sirups.

Existing tables for various sugars, covering Baumé–dry substance, are not applicable to corn sirup. One publication (*7*) covers a small range of Baumés. Various references are to be found in the trade journals covering a specific Baumé, usually based on a private table of one refiner. The industry has lacked uniform Baumé–dry substance tables primarily for two reasons: (1) The amount of dry substance for a given Baumé increases with increasing dextrose equivalent, and (2) there was considerable dispute in the industry itself regarding moisture methods and the stability of the specific product on drying. Through an extended research program, the questions of methods and stability have been solved and these methods accepted by the industry and published (*3–8*).

Since commercial corn sirups are extremely viscous, it is difficult to obtain satisfactory readings with a Baumé hydrometer at ordinary temperatures. To eliminate this difficulty an arbitrary method was adopted years ago and is still in use. It consists of making the determination at 140° F. (60° C.) using a hydrometer standardized at 60° F., adding the arbitrary correction of 1.00° Baumé to the observed reading. The application of this correction approximately corrects to the reading at 100° F. The hydrometer used is that based on 145 modulus, 60° F. (the standard in the United States for liquids heavier than water). Thus commercial Baumé, the basis for sale, is designated as Baumé=Baumé 140°/60° F. + 1.00. The use of a hydrometer at 80° F. above the temperature of standardization requires special considerations which will be discussed in detail.

When the temperature of observation differs from that at which the hydrometer was standardized—i. e., the normal reading—the observed reading is not the true specific gravity according to the basis of the instrument, but a figure which differs from the normal reading by an amount depending upon the difference in temperature and the relative thermal expansion of the instrument and of the particular liquid. A table of temperature corrections of considerable precision may be determined, provided subsequent hydrometers are made with glass of the same thermal expansion and of the same physical dimensions as the primary standard. The Baumés in the following tables are based on readings made with the so-called "streamlined" type hydrometers, having a range of 10.00° Bé. graduated to 0.1° Bé. Hydrometers of such simple design have definite advantages when used at temperatures higher than that of standardization, particularly in viscous liquids.

Determination of Baumé

The procedure for determination of Baumé on corn sirup requires that the test be made at 140° F. This temperature materially reduces the viscosity of the sirup, but introduces other troubles.

EVAPORATION. Surface evaporation is the principal difficulty. At a temperature of 140° F., water is lost rapidly by evaporation at the surface. As water is lost, a tough surface skin develops, which must be removed before the hydrometer is introduced. Even so, a small amount of skin will develop during the reading of the hydrometer. This exerts a drag on the free movement of the hydrometer and its effect must be removed before the reading is made. Two common methods are employed to alleviate this trouble, both of which are only partially successful:

1. The skin may be removed from the hydrometer stem by means of a thin straw, such as a broom straw, or a thin wire.
2. The skin effect on the hydrometer is retarded or minimized by diluting the surface sirup around the stem of the hydrometer. A capillary tube is used to place small drops of water around the stem.

While these methods help, particularly when the meniscus is read from above, they cause striations through density differences, or a blurred reading when the hydrometers are read through the glass at plane surface level, as is required for more accurate work.

CONDENSATION. When the sirup under test is placed in a cylinder which is not completely filled (in order to allow for added volume when the hydrometer is immersed), water vapor condenses on the inner surfaces of the cylinder and subsequently runs down into the sirup. This produces a change in density, causing density striations which give blurred readings when the hydrometer scale is read through the glass cylinder at plane surface line.

TIME FOR SIRUP TO ATTAIN BATH TEMPERATURE. A correct Baumé reading is obtainable only after the temperature of the

sirup and hydrometer equals that of the bath—that is, 140° F. An error is often introduced by reading the Baumé before sufficient time has elapsed for the temperature of the sirup and hydrometer to attain bath temperature. A study has been made of the time necessary (for sirup at 70° F.) to attain bath temperature. The results are shown in Table I. When glass cylinders are used, a longer time is required.

Effect of Air. The presence of air in corn sirup or corn sugar sirup results in too low a Baumé reading. A serious error is often introduced, as the observer fails to recognize the importance of air on the reading. Sirup must be free of air before an accurate reading can be obtained. The time required to remove all the air is longer than generally assumed, and in the case of copper cylinders is difficult to observe. In order to obtain a measure of the time necessary to remove all the air from the sirup in a cylinder, the following experiment was made:

Corn sirups (42 D. E.) of different Baumés were poured with average care into glass cylinders 2 inches in inside diameter and the cylinders were placed in a water bath at 140° F. The observed time required for all the air trapped in the pouring operation to float to the surface where it could be removed by skimming was as follows:

° *Bé.*	*Hours*
42	1
43	3.5
44	6
45	24
46	95

The effect of small residual quantities of trapped air is too often discounted as insignificant. In order to determine the magnitude of this effect, the following test was made:

Corn sirups (42 D. E.) of different Baumés were poured into glass cylinders, which were placed in a water bath at 140° F. When the temperature in all the cylinders reached 140° F., a stream of air was introduced through a capillary for an equal period of time in all cylinders, after which the Baumé was read at stated intervals and the amount of deviation between the observed reading and that on the final air-free sirup determined. (Table II).

These data show the danger of introducing errors through occluded air, since an analyst, noting that the reading on the 46° Bé. sirup had been constant for 60 minutes, would assume constancy and report a false reading. Apparently in sirups of such high viscosity, the effect of small amounts of residual air is more slowly transmitted to the hydrometer, for in cross checks between laboratories on such samples there is always better agreement, although the data are invariably low.

Effect of Grease, etc., on Hydrometer Reading. Very small traces of grease, etc., have a noticeable effect on the hydrometer position, particularly in the range of 35° to 43° Bé. Errors caused by surface effects can be eliminated in many liquids through the use of an overflow (*2*) which renews the surface of the liquid, but this is not practical in the case of corn sirup. At least, the cylinder and hydrometer used can be thoroughly cleaned by sulfuric acid–sodium bichromate mixture, and the hydrometer can be kept clean and free from grease by touching only the portion of the stem above the scale during manipulation.

Table I. Time to Attain Bath Temperature

Copper Cylinder	Commercial Baumé of Corn Sirup 42°	43°	44°	45°	46°
Inches	Time to Reach 140° F. *Minutes*				
2	55	70	80	90	100
2.25	72	86	98	111	124
2.5	90	107	120	136	152
3	130	155	180	205	240

Table II. Deviation

Time	Deviation from True Baumé Reading 42°	43°	44°	46°
Min.	° *Bé.*	° *Bé.*	° *Bé.*	° *Bé.*
10	−0.14	−0.16	−0.28	−0.15
30	−0.07	−0.07	−0.17	−0.15
60	−0.02	−0.05	−0.07	−0.15
120	−0.00	−0.01	−0.02	−0.07
1010	....	....	....	−0.03

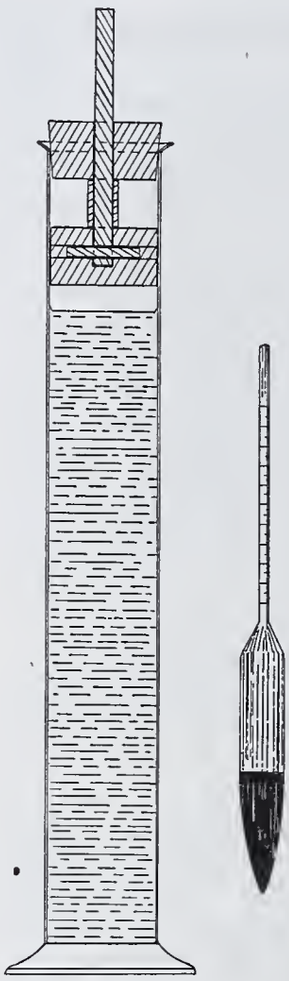

Figure 1. Hydrometer and Special Cylinder

Although the difficulties in the accurate determination of Baumé in corn sirup are many, it is believed that the official referee method adopted by the Corn Industries Research Foundation overcomes them to a large extent.

C. I. R. F. Official Referee Method for Baumé

The necessary equipment is shown in Figure 1. The important step in the procedure is the method of sealing the sample in the cylinder, during the period of heating to 140° F. and of removing all the air. The equipment for this consists of two rubber stoppers which fit snugly in the cylinder, and are separated on a metal rod by approximately 3 inches. The rod is fixed in the bottom stopper, but does not extend through it. The top stopper is free to move on the rod, although sufficiently tight to maintain a predetermined position. Through the use of these stoppers, little or no water evaporates from the surface and cool air does not reach the inner walls of the cylinder, thus enabling a given sample of sirup to remain in the bath without the difficulties previously mentioned.

Apparatus. Water bath, 32-gallon capacity, lagged, equipped with stirrer and metastatic mercury regulators with sensitivity of 0.02° C., set at 100° and 140° F. The bath was further equipped with a live steam inlet to raise the temperature rapidly from 60° to 100° to 140° F.

Vacuum ovens, Weber, with Cenco Megavac pumps, producing an oven pressure of less than 1 mm.

Distillation apparatus, as described (*5*, *7*, *9*). Moisture apparatus, as described (*3*). Pycnometers, as shown. Hydrometer cylinders, 15 × 2.25 inch, Pyrex, without lip. Hydrometers, 10.00° Bé., graduated to 0.1° Bé.

The Corn Industries Research Foundation has adopted specifications for referee hydrometers to be used by the industry in the sale of corn sirup. These specifications conform to those of the hydrometers used in the preparation of the tables appearing here and are as follows:

The hydrometers shall be the streamlined type, 145 modulus, standardized at 60° F., with a range of 10.00° Bé., graduated to 0.1° Bé., manufactured from Kimble flint glass with a linear coefficient of expansion of 0.0000092.

The over-all length of the hydrometer shall be not more than 13 or less than 12 inches. The diameter of the body at the center shall be not more than 0.790 or less than 0.770 inch.

For 35° to 45° Baumé hydrometers, the scale length for 10 Baumé degrees shall not be more than 155 mm. or less than 147 mm. For other hydrometers, the scale length for 10 Baumé degrees shall not be more than 140 mm. or less than 130 mm. Hydrometers to these specifications are made and stocked by Wm. Hiergesell & Sons, New York, N. Y., as C. I. R. F. hydrometers.

Procedure. Pour the sirup under test into the cylinder within 4 inches of the top, taking care that the sides are free of sirup. Seal the cylinder with the dual stoppers, the bottom stopper being placed within 0.5 inch of the sirup surface and the top stopper closing the cylinder. Place the cylinder in the water

Table III. Expansion of Water

Temperature, ° C.	Specific Gravity of Water, $T°/4°$ C.	Specific Gravity of Water, $T°/15.56°$ C.	Baumé of Water, $T°/15.56°$ C.
15.56	0.99904	1.00000	0.000
37.78	0.99307	0.99402	−0.872
60.00	0.98324	0.98418	−2.331

Table IV. Specific Gravities

Temperature, ° C.	Specific Gravity Apparent	Specific Gravity Corrected	Baumé, Pycnometer 6 Apparent	Baumé, Pycnometer 6 Corrected
15.56/15.56	1.00000	1.00000	0.000	0.000
37.78/15.56	0.99423	0.99402	−0.842	−0.872
60.00/15.56	0.98465	0.98424	−2.260	−2.322
			Pycnometer 7	
15.56/15.56	1.00000	1.00000	0.000	0.000
37.78/15.56	0.99423	0.99402	−0.842	−0.872
60.00/15.56	0.98457	0.98415	−2.272	−2.335

bath so that the water extends to within 1 inch of the top. At the same time, run distilled water into a second cylinder, place the test hydrometer in it, and place in the water bath. When the sirup is free of air, place the cylinder on a higher shelf, so that the surface of the sirup will extend about 0.5 inch above the water after immersing the hydrometer. Remove dual stoppers from the cylinder. Remove test hydrometer by contact with top of the stem, dry quickly with clean towel, and immerse in the sirup.

In approximately 10 minutes read the hydrometer at intervals of 2 minutes through the cylinder, taking every care to avoid parallax. Read until two or more readings are identical. Watch carefully that the sirup meniscus is uniform around the hydrometer stem. Usually it is necessary to add a very small drop of water at the stem meniscus. To the observed reading of the hydrometer add any correction found necessary from the previous standardization against a Bureau of Standards hydrometer.

Baumé by Pycnometers

In the initial work Baumés were determined by hydrometers as given above. Hydrometers were compared with an instrument certified by the Bureau of Standards at five points. The Baumé reading on distilled water was −0.71° at 100° F., and −2.01° at 140° F. The Baumé on a given sirup was the average of the readings of two or more observers. In view of the large amount of data necessary for complete tables, this method was so laborious and tedious that the determination of Baumé was transferred from hydrometers to pycnometers, whereby two or more could be employed on a single sirup by one analyst, and greater accuracy could be obtained.

The initial work was started with the usual type of flat-bottomed pycnometers, but it was found that when used at elevated temperatures, each expanded differently according to some small change in design. Accordingly, a new type of pycnometer was designed in the form of a sphere with standard taper joints of Pyrex, as shown in Figure 2.

These pycnometers agreed among themselves when used at elevated temperatures, and specific gravities at 100°/60° F. and 140°/60° F. were close to the true specific gravity after correction for the expansion of glass, as will be seen from the following tables. Table III gives the theoretical expansion of water itself, specific gravity being calculated on the basis of grams per milliliter.

Table IV gives the data obtained by two typical pycnometers, showing the specific gravities, as is or apparent, and after correction for the cubical expansion of Pyrex (0.0000096 per ° C.).

The Baumé readings obtained by the Pyrex pycnometers did not check the values of hydrometers for distilled water at 37.78° and 60.00° C. As the glass in the two instruments was different, this was at first explained on the basis of difference in thermal expansion.

A formula was developed whereby the apparent specific gravity of pycnometers could be translated to hydrometer readings. The basis for the formula was the hydrometer readings in distilled water at 37.78° C. (−0.71° Bé.) and 60.00° C. (−2.01° Bé.), as follows:

$$H = \frac{W_2 + (A \times \bar{V} \times C)}{W_1}$$

when

H = specific gravity at $T°/15.56°$ C. (in vacuum) corresponding to hydrometer reading at $T°$

W_2 = weight of liquid in pycnometer at $T°$, corrected to vacuum

W_1 = weight of water in pycnometer at 15.56° C., corrected to vacuum

A = $\frac{W_2}{W_1}$ apparent specific gravity

V = volume of pycnometer at 15.56° C.

C = correction

or

$$C = \frac{W_1H - W_2}{AV}$$

The seven pycnometers used had an average capacity of 80 ml. The average value for C at 37.78° C. was 0.00089 and at 60° C. was 0.00175.

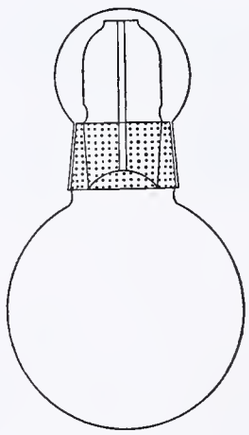

Figure 2. Pycnometer

The apparent specific gravities by pycnometers on various concentrations of corn sirup, employing the correction as indicated above, were compared with actual readings by hydrometers in the same sirup and found to agree within 0.01° Bé. The same relationship was found true, employing sulfuric acid solutions at the temperatures indicated.

In Tables V to XVI, the Baumé recorded is in all cases the hydrometer reading at the temperature indicated. The specific gravity in air is the corresponding value of the Baumé. In the case of the 60°/60° F. tables, the Baumé reading is true, as is also the specific gravity in air. However, in the tables at 100°/60° F. and 140°/60° F., the Baumé reading and the assigned specific gravity are apparent. True specific gravity—vacuum—can be obtained for Baumé readings at the three table temperatures by means of Table V, which covers the corrections to be subtracted from the assigned specific gravity in air.

Determination of Moisture

Corn sirup is extremely stable at the temperatures encountered in the usual moisture determination methods. It can be dried without decomposition as high as 112° C., if the pH of the product is within the range of 4.5 to 5.5. The basis for this statement is that identical values can be obtained by benzene distillation (80–81° C.), vacuum oven drying (100° C.), and toluene distillation (112° C.), and the results of these determinations can be correlated to others obtained at much lower temperatures through special methods (*3*, *8*).

In a moisture determination, the problem involved is that of spreading the corn sirup over a sufficiently large surface so that the moisture can be removed quickly and completely (*3*, *7*, *8*, *9*).

Crude corn sugars, in contrast to corn sirups, are more variable among manufacturers, and are marketed with several dextrose equivalents and ash contents. They are more sus-

TABLE V. CORRECTIONS

Hydrometer Reading	Assigned Specific Gravity, Air	Corrections to Be Subtracted: Air to vacuum	Expansion of glass	Hydrometer correction	Total	True Specific Gravity Vacuum
		60°/60° F.				
0.00	1.0000	0.00000	.....	.....	0.0000	1.0000
5.00	1.0358	0.00005	.....	.....	0.0001	1.0357
10.00	1.0742	0.00012	.....	.....	0.0001	1.0741
15.00	1.1156	0.00019	.....	.....	0.0002	1.1154
20.00	1.1602	0.00025	.....	.....	0.0002	1.1600
25.00	1.2086	0.00031	.....	.....	0.0003	1.2083
30.00	1.2612	0.00037	.....	.....	0.0003	1.2609
35.00	1.3186	0.00043	.....	.....	0.0004	1.3182
40.00	1.3815	0.00050	.....	.....	0.0005	1.3810
45.00	1.4506	0.00056	.....	.....	0.0006	1.4500
		100°/60° F.				
−0.71	0.9951	−0.00001	0.00021	0.00085	0.00105	0.9940
0.00	1.0000	0.00000	0.00021	0.00090	0.00111	0.9989
5.00	1.0358	0.00005	0.00022	0.00093	0.00120	1.0346
10.00	1.0742	0.00012	0.00023	0.00095	0.00130	1.0729
15.00	1.1156	0.00019	0.00024	0.00100	0.00143	1.1142
20.00	1.1602	0.00025	0.00025	0.00104	0.00154	1.1587
25.00	1.2086	0.00031	0.00026	0.00108	0.00165	1.2069
30.00	1.2612	0.00037	0.00027	0.00113	0.00177	1.2594
35.00	1.3186	0.00043	0.00028	0.00117	0.00188	1.3167
40.00	1.3815	0.00050	0.00030	0.00120	0.00200	1.3795
45.00	1.4506	0.00056	0.00031	0.00124	0.00211	1.4485
		140°/60° F.				
−2.01	0.9864	−0.00002	0.00042	0.00166	0.00206	0.9843
0.00	1.0000	0.00000	0.00042	0.00174	0.00216	0.9978
5.00	1.0358	0.00005	0.00044	0.00184	0.00233	1.0335
10.00	1.0742	0.00012	0.00046	0.00191	0.00249	1.0717
15.00	1.1156	0.00019	0.00047	0.00199	0.00265	1.1129
20.00	1.1602	0.00025	0.00050	0.00206	0.00281	1.1574
25.00	1.2086	0.00031	0.00052	0.00215	0.00298	1.2056
30.00	1.2612	0.00037	0.00055	0.00222	0.00314	1.2581
35.00	1.3186	0.00043	0.00057	0.00230	0.00330	1.3153
40.00	1.3815	0.00050	0.00059	0.00238	0.00347	1.3780
45.00	1.4506	0.00056	0.00061	0.00246	0.00363	1.4470

ceptible to decomposition because of a larger protein, acid, and ash content. Further, as the dextrose equivalent of the starch hydrolysate increases, the material retains its water more tenaciously. This appears to reach a peak at about 82 D. E. Thus the problem of drying has been to remove these last traces of residual moisture, without resorting to high temperatures which lead to decomposition. Occasionally with some lots of crude sugar, the vacuum oven procedure described gave results which made the distinction between the last traces of water removal and decomposition a difficult decision. In such cases benzene distillation was employed as confirmative. The basis for this conclusion is described elsewhere (*5*, *6*).

The determination of moisture in corn sirup and corn sugar is discussed fully elsewhere (*3–9*). The methods used were as follows:

CORN SIRUP. Filter-Cel method (diatomaceous silica, Johns Manville Hy-Flo), vacuum oven at 100° C.; alternative methods, toluene distillation and benzene distillation.

CORN SUGAR. For 80 to 92 D. E., Filter-Cel method, vacuum oven at 80° C.; alternative, benzene distillation.

PREPARATION OF SAMPLE. The sirups used for the tables were from two factories. The heavy sirups or solid sugars were diluted with distilled water, refined with activated carbon, and either diluted further or concentrated under vacuum to the desired density. The warm sirup was run into a 2-liter round-bottomed flask fitted with rubber stopper and stopcock. The flask was then evacuated to a pressure of 70 to 75 mm. (mercury) by a water pump and slowly cooled to 80° to 90° F. During the cooling process the sirup was constantly stirred by rotating the flask. A homogeneous cooled sample substantially free from dissolved air was obtained by this procedure. The cold sample was transferred carefully to 4-ounce glass bottles, fitted with

TABLE VI. EXPERIMENTAL BÉ.–D. E.–D. S.

(140°/60°. F = Factor = D. S./Bé. + 2.01)

Baumé	42.00 D. E. F	42.00 D. E. D. S.	55.00 D. E. F	55.00 D. E. D. S.	89.00 D. E. F	89.00 D. E. D. S.	90.7 D. E. F	90.7 D. E. D. S.
−1.00	1.7469	1.76	1.7553	1.78	1.7830	1.80	1.7823	1.80
0.00	1.7479	3.51	1.7566	3.53	1.7845	3.59	1.7838	3.59
1.00	1.7489	5.26	1.7579	5.29	1.7861	5.38	1.7854	5.37
2.00	1.7499	7.02	1.7593	7.06	1.7876	7.17	1.7870	7.17
3.00	1.7510	8.77	1.7607	8.82	1.7893	8.96	1.7886	8.96
4.00	1.7521	10.53	1.7619	10.59	1.7909	10.76	1.7901	10.76
5.00	1.7532	12.29	1.7633	12.36	1.7925	12.57	1.7917	12.56
6.00	1.7543	14.05	1.7647	14.14	1.7942	14.37	1.7934	14.37
7.00	1.7554	15.82	1.7661	15.91	1.7959	16.18	1.7951	16.17
8.00	1.7565	17.58	1.7675	17.69	1.7977	18.00	1.7968	17.99
9.00	1.7577	19.35	1.7689	19.48	1.7994	19.81	1.7985	19.80
10.00	1.7589	21.12	1.7704	21.26	1.8012	21.63	1.8003	21.65
11.00	1.7601	22.90	1.7719	23.05	1.8031	23.46	1.8021	23.42
12.00	1.7614	24.68	1.7733	24.84	1.8050	25.29	1.8040	25.28
13.00	1.7627	26.46	1.7749	26.64	1.8070	27.12	1.8058	27.11
14.00	1.7640	28.24	1.7764	28.44	1.8090	28.96	1.8077	28.94
15.00	1.7654	30.03	1.7780	30.24	1.8110	30.80	1.8097	30.78
16.00	1.7668	31.82	1.7796	32.05	1.8131	32.65	1.8117	32.63
17.00	1.7682	33.61	1.7813	33.86	1.8152	34.51	1.8138	34.48
18.00	1.7697	35.41	1.7830	35.68	1.8174	36.37	1.8159	36.34
19.00	1.7711	37.21	1.7847	37.50	1.8196	38.23	1.8181	38.20
20.00	1.7727	39.02	1.7865	39.32	1.8220	40.10	1.8203	40.06
21.00	1.7742	40.82	1.7883	41.15	1.8244	41.98	1.8227	41.94
22.00	1.7758	42.64	1.7902	42.98	1.8268	43.86	1.8250	43.82
23.00	1.7775	44.46	1.7921	44.82	1.8294	45.75	1.8275	45.71
24.00	1.7793	46.28	1.7940	46.66	1.8320	47.65	1.8300	47.60
25.00	1.7810	48.10	1.7960	48.51	1.8347	49.56	1.8326	49.50
26.00	1.7829	49.94	1.7981	50.37	1.8375	51.47	1.8354	51.41
27.00	1.7848	51.78	1.8003	52.23	1.8404	53.39	1.8382	53.33
28.00	1.7867	53.62	1.8025	54.09	1.8433	55.32	1.8411	55.25
29.00	1.7887	55.47	1.8048	55.97	1.8464	57.26	1.8441	57.19
30.00	1.7908	57.32	1.8071	57.85	1.8496	59.21	1.8472	59.13
31.00	1.7930	59.18	1.8095	59.73	1.8529	61.16	1.8505	61.09
32.00	1.7952	61.05	1.8120	61.63	1.8563	63.13	1.8538	63.05
33.00	1.7975	62.93	1.8146	63.53	1.8598	65.11	1.8572	65.02
34.00	1.7999	64.81	1.8173	65.44	1.8634	67.10	1.8607	67.00
35.00	1.8024	66.71	1.8201	67.36	1.8672	69.11	1.8644	69.00
36.00	1.8051	68.61	1.8231	69.30	1.8710	71.12	1.8681	71.01
37.00	1.8078	70.52	1.8261	71.24	1.8750	73.14	1.8720	73.03
38.00	1.8107	72.45	1.8293	73.19	1.8791	75.18	1.8760	75.06
39.00	1.8137	74.38	1.8326	75.16	1.8834	77.24	1.8801	77.10
40.00	1.8169	76.33	1.8361	77.14	1.8877	79.30	1.8845	79.17
41.00	1.8202	78.29	1.8397	79.13	1.8922	81.38	1.8890	81.25
42.00	1.8237	80.26	1.8436	81.14	1.8968	83.48	1.8935	83.33
43.00	1.8273	82.25	1.8477	83.17	1.9017	85.60	1.8982	85.44
44.00	1.8311	84.25	1.8518	85.20	1.9066	87.72	1.9030	87.56
45.00	1.8350	86.26	1.8562	87.26	1.9116	89.86	1.9079	89.69
46.00	1.8391	88.30	1.8609	89.34	1.9168	92.03	1.9130	91.84

TABLE VII. EXPERIMENTAL BÉ.–D. E.–D. S.

(100°/60°. F = Factor = D. S./Bé. +0.71)

Baumé	42.00 D. E. F	42.00 D. E. D. S.	55.00 D. E. F	55.00 D. E. D. S.	89.00 D. E. F	89.00 D. E. D. S.	90.7 D. E. F	90.7 D. E. D. S.
0.00	1.7544	1.25	1.7680	1.26	1.7927	1.27	1.7898	1.27
1.00	1.7555	3.00	1.7692	3.03	1.7943	3.07	1.7914	3.06
2.00	1.7567	4.76	1.7704	4.80	1.7959	4.87	1.7931	4.86
3.00	1.7579	6.52	1.7716	6.57	1.7976	6.67	1.7948	6.66
4.00	1.7591	8.29	1.7729	8.35	1.7993	8.47	1.7966	8.46
5.00	1.7604	10.05	1.7741	10.13	1.8010	10.28	1.7984	10.27
6.00	1.7617	11.82	1.7755	11.91	1.8028	12.09	1.8002	12.08
7.00	1.7630	13.59	1.7768	13.70	1.8046	13.91	1.8020	13.89
8.00	1.7643	15.37	1.7782	15.49	1.8065	15.73	1.8039	15.71
9.00	1.7657	17.15	1.7796	17.28	1.8084	17.56	1.8058	17.53
10.00	1.7671	18.93	1.7810	19.07	1.8104	19.39	1.8078	19.36
11.00	1.7685	20.71	1.7825	20.87	1.8125	21.22	1.8098	21.19
12.00	1.7699	22.50	1.7840	22.67	1.8145	23.06	1.8119	23.03
13.00	1.7713	24.28	1.7855	24.48	1.8165	24.90	1.8140	24.87
14.00	1.7728	26.08	1.7871	26.29	1.8187	26.75	1.8162	26.72
15.00	1.7743	27.88	1.7888	28.10	1.8209	28.61	1.8184	28.57
16.00	1.7759	29.68	1.7904	29.92	1.8231	30.47	1.8207	30.42
17.00	1.7775	31.48	1.7921	31.74	1.8254	32.33	1.8230	32.28
18.00	1.7791	33.29	1.7939	33.56	1.8278	34.20	1.8253	34.15
19.00	1.7808	35.10	1.7957	35.39	1.8302	36.07	1.8277	36.02
20.00	1.7825	36.92	1.7975	37.22	1.8327	37.96	1.8302	37.90
21.00	1.7843	38.74	1.7994	39.06	1.8353	39.85	1.8328	39.79
22.00	1.7861	40.56	1.8014	40.91	1.8379	41.74	1.8354	41.68
23.00	1.7879	42.39	1.8034	42.76	1.8407	43.64	1.8381	43.58
24.00	1.7898	44.23	1.8055	44.61	1.8435	45.55	1.8409	45.49
25.00	1.7918	46.07	1.8077	46.47	1.8464	47.47	1.8437	47.40
26.00	1.7938	47.91	1.8099	48.34	1.8494	49.40	1.8466	49.32
27.00	1.7960	49.77	1.8122	50.22	1.8525	51.33	1.8497	51.25
28.00	1.7982	51.63	1.8146	52.10	1.8556	53.27	1.8528	53.19
29.00	1.8005	53.49	1.8171	53.99	1.8589	55.23	1.8560	55.14
30.00	1.8028	55.36	1.8196	55.88	1.8623	57.19	1.8593	57.10
31.00	1.8052	57.24	1.8223	57.79	1.8658	59.16	1.8626	59.06
32.00	1.8077	59.13	1.8250	59.70	1.8694	61.15	1.8661	61.04
33.00	1.8104	61.03	1.8279	61.62	1.8731	63.14	1.8697	63.03
34.00	1.8131	62.93	1.8308	63.55	1.8769	65.15	1.8735	65.03
35.00	1.8159	64.85	1.8338	65.49	1.8809	67.17	1.8774	67.04
36.00	1.8189	66.77	1.8370	67.44	1.8850	69.20	1.8814	69.07
37.00	1.8219	68.70	1.8403	69.40	1.8893	71.25	1.8855	71.10
38.00	1.8251	70.65	1.8438	71.37	1.8936	73.30	1.8898	73.15
39.00	1.8283	72.60	1.8474	73.36	1.8981	75.37	1.8943	75.22
40.00	1.8317	74.57	1.8512	75.36	1.9028	77.46	1.8990	77.31
41.00	1.8353	76.55	1.8552	77.38	1.9078	79.57	1.9038	79.41
42.00	1.8391	78.55	1.8594	79.41	1.9129	81.70	1.9088	81.53
43.00	1.8430	80.56	1.8637	81.46	1.9182	83.84	1.9140	83.67
44.00	1.8470	82.58	1.8682	83.53	1.9236	86.00	1.9194	85.82
45.00	1.8512	84.62	1.8728	85.61	1.9292	88.18	1.9250	87.99
46.00	1.8556	86.68	1.8776	87.70	1.9350	90.38	1.9307	90.18
47.00	1.8602	88.75	1.8826	89.82	1.9410	92.61	1.9367	92.40

TABLE VIII. EXPERIMENTAL BÉ.–D. E.–D. S.

(60°/60°. F = Factor = D. S./Bé.)

Baumé	42.00 D. E. F	42.00 D. E. D. S.	55.00 D. E. F	55.00 D. E. D. S.	89.00 D. E. F	89.00 D. E. D. S.	90.7 D. E. F	90.7 D. E. D. S.
0.00		0.00		0.00		0.00		0.00
1.00	1.7358	1.74	1.7407	1.74	1.7670	1.77	1.7638	1.76
2.00	1.7371	3.47	1.7425	3.48	1.7690	3.54	1.7658	3.53
3.00	1.7385	5.21	1.7443	5.23	1.7710	5.31	1.7678	5.30
4.00	1.7399	6.96	1.7462	6.98	1.7730	7.09	1.7699	7.08
5.00	1.7413	8.71	1.7480	8.74	1.7750	8.87	1.7720	8.86
6.00	1.7427	10.46	1.7499	10.50	1.7771	10.66	1.7742	10.64
7.00	1.7441	12.21	1.7518	12.26	1.7793	12.46	1.7764	12.43
8.00	1.7456	13.96	1.7537	14.03	1.7816	14.25	1.7787	14.23
9.00	1.7471	15.72	1.7557	15.80	1.7838	16.05	1.7810	16.03
10.00	1.7487	17.48	1.7577	17.58	1.7861	17.86	1.7833	17.83
11.00	1.7503	19.25	1.7597	19.36	1.7885	19.67	1.7857	19.64
12.00	1.7519	21.02	1.7617	21.14	1.7909	21.49	1.7881	21.46
13.00	1.7536	22.80	1.7638	22.93	1.7934	23.31	1.7907	23.28
14.00	1.7553	24.58	1.7659	24.72	1.7959	25.14	1.7933	25.11
15.00	1.7571	26.36	1.7680	26.52	1.7985	26.98	1.7959	26.94
16.00	1.7589	28.14	1.7701	28.32	1.8011	28.82	1.7986	28.78
17.00	1.7608	29.93	1.7723	30.13	1.8038	30.66	1.8013	30.62
18.00	1.7627	31.73	1.7746	31.94	1.8066	32.52	1.8040	32.47
19.00	1.7646	33.53	1.7769	33.76	1.8094	34.38	1.8068	34.33
20.00	1.7666	35.33	1.7792	35.58	1.8123	36.25	1.8097	36.19
21.00	1.7687	37.14	1.7816	37.41	1.8153	38.12	1.8127	38.07
22.00	1.7708	38.96	1.7840	39.25	1.8184	40.00	1.8158	39.95
23.00	1.7730	40.78	1.7865	41.09	1.8215	41.89	1.8189	41.83
24.00	1.7752	42.60	1.7890	42.94	1.8247	43.79	1.8221	43.73
25.00	1.7775	44.44	1.7916	44.79	1.8281	45.70	1.8254	45.64
26.00	1.7799	46.28	1.7943	46.65	1.8315	47.62	1.8288	47.55
27.00	1.7823	48.12	1.7970	48.52	1.8350	49.55	1.8323	49.47
28.00	1.7848	49.97	1.7998	50.39	1.8387	51.48	1.8360	51.41
29.00	1.7874	51.83	1.8027	52.28	1.8424	53.43	1.8397	53.35
30.00	1.7901	53.70	1.8057	54.17	1.8464	55.39	1.8435	55.31
31.00	1.7929	55.58	1.8088	56.07	1.8504	57.36	1.8474	57.27
32.00	1.7958	57.47	1.8119	57.98	1.8545	59.34	1.8515	59.25
33.00	1.7987	59.36	1.8152	59.90	1.8588	61.34	1.8557	61.24
34.00	1.8018	61.26	1.8186	61.83	1.8633	63.35	1.8601	63.24
35.00	1.8051	63.18	1.8221	63.77	1.8679	65.38	1.8646	65.26
36.00	1.8084	65.10	1.8258	65.73	1.8727	67.42	1.8692	67.29
37.00	1.8119	67.04	1.8296	67.70	1.8775	69.47	1.8740	69.34
38.00	1.8155	68.99	1.8335	69.67	1.8825	71.54	1.8790	71.40
39.00	1.8192	70.95	1.8377	71.67	1.8877	73.62	1.8841	73.48
40.00	1.8231	72.92	1.8420	73.68	1.8931	75.73	1.8893	75.57
41.00	1.8272	74.92	1.8466	75.71	1.8987	77.85	1.8946	77.68
42.00	1.8314	76.92	1.8514	77.76	1.9045	79.99	1.9002	79.81
43.00	1.8359	78.94	1.8564	79.83	1.9105	82.15	1.9061	81.96
44.00	1.8406	80.99	1.8616	81.91	1.9167	84.33	1.9121	84.13
45.00	1.8456	83.05	1.8670	84.01	1.9229	86.53	1.9183	86.32
46.00	1.8509	85.14	1.8726	86.14	1.9293	88.75	1.9246	88.53
47.00	1.8564	87.25	1.8784	88.28	1.9359	90.99	1.9310	90.76
48.00	1.8621	89.38	1.8843	90.45	1.9426	93.24	1.9375	93.00

screw tops. Ten bottles were so prepared. One bottle was used for each Baumé and each moisture determination. The specific gravity determination was the average of two or three tests, the variation never being more than one in the fourth decimal place. The moisture determinations were the average of two to six tests by different methods and carried out by two analysts. The maximum variation between analysts was 0.03 per cent with an average of 0.015 per cent.

Dextrose Equivalent and Ash

Dextrose equivalent is the amount of reducing sugars, determined by the Lane-Eynon (*10*) method, expressed as dextrose on the dry substance obtained. The ash, largely sodium chloride, was determined by ashing at dull red heat according to the A. O. A. C. procedure (*1*). The results were in agreement with direct determination of chlorides by the Volhard titration.

PROCEDURE. In this work one chemist determined all the specific gravities; one or two, the moistures. With sirups which would crystallize, tests were started as soon as the samples were prepared. The dextrose equivalent was the average of tests made by at least two chemists.

The Baumé–dry substance correlation was made on four sirups as follows:

D. E.	Ash, D. S. B.
42.00	0.28
55.00	0.30
89.00	0.61
90.7	1.22

The first two corn sirups are typical for all manufacturers in dextrose equivalent, specific rotation, and ash content. The latter two are typical of two general types of crude sugar manufactured in the industry—a low-ash sugar of approximately 89 D. E. maximum and a high-ash sugar of approximately 92 D. E. maximum.

The complete range of Baumé 0 to 47° was covered at three temperatures—60.0°, 100.0°, and 140.0° F. The points were determined every 5° Bé. in the lower ranges and every 2° Bé. in the higher ranges. The average number of points for each curve was nineteen. The method of plotting used at first was Baumé against dry substance. The paper chosen was millimeter paper, each millimeter equaling 0.10° Bé. and 0.10 per cent dry substance. The second decimal place was plotted on the paper by the fine point of the drawing instrument and a hand lens. Although this was satisfactory for the Baumé, the length of the curve for dry substance was so great that it did not sufficiently show up small variations in dry substance. The method was changed to one of factors against Baumé. The factors were determined as follows:

$$\text{At } 60^\circ \text{ F., factor } 60^\circ \text{ F.} = \frac{\text{D. S.}}{\text{Baumé}}$$

$$\text{At } 100^\circ \text{ F., factor } 100^\circ \text{ F.} = \frac{\text{D. S.}}{\text{Baumé} + 0.71}$$

$$\text{At } 140^\circ \text{ F., factor } 140^\circ \text{ F.} = \frac{\text{D. S.}}{\text{Baumé} + 2.01}$$

The use of these factors shortened the ordinate and amplified the experimental variation. Thus at 5° Bé. each millimeter equaled 0.0090 per cent D. S. The curve was drawn through the points by means of a steel ferule and a very fine-pointed hard pencil. From the curve so drawn, the factors were read for each Baumé and, in turn, the dry substance value was obtained. The values so obtained were in excellent agreement with experimental results.

The computed data for the three temperatures appear in Tables VI, VII, and VIII.

TABLE IX. EXPANDED BÉ.–D. E.–D. S.

(140°/60°)

D. E.	30.00	42.00	55.00	82.00	87.00	89.00	91.2	90.7
Ash	0.28	0.28	0.30	0.41	0.61	0.61	0.61	1.22
Baumé	Per Cent Dry Substance							
−1.00	1.75	1.76	1.78	1.80	1.80	1.80	1.80	1.80
0.00	3.49	3.51	3.53	3.58	3.58	3.59	3.59	3.59
1.00	5.23	5.26	5.29	5.36	5.37	5.38	5.39	5.37
2.00	6.98	7.02	7.06	7.15	7.17	7.17	7.18	7.17
3.00	8.72	8.77	8.82	8.94	8.96	8.96	8.97	8.96
4.00	10.47	10.53	10.59	10.73	10.76	10.76	10.77	10.76
5.00	12.22	12.29	12.36	12.53	12.56	12.57	12.58	12.56
6.00	13.97	14.05	14.14	14.33	14.36	14.37	14.39	14.37
7.00	15.73	15.82	15.91	16.13	16.17	16.18	16.20	16.17
8.00	17.48	17.58	17.69	17.94	17.98	18.00	18.02	17.98
9.00	19.24	19.35	19.48	19.75	19.79	19.81	19.83	19.80
10.00	20.99	21.12	21.26	21.56	21.61	21.63	21.65	21.62
11.00	22.76	22.90	23.05	23.38	23.44	23.46	23.48	23.45
12.00	24.52	24.68	24.84	25.20	25.26	25.29	25.31	25.27
13.00	26.29	26.46	26.64	27.02	27.09	27.12	27.15	27.10
14.00	28.06	28.24	28.44	28.85	28.93	28.96	28.99	28.94
15.00	29.83	30.03	30.24	30.69	30.77	30.80	30.84	30.78
16.00	31.61	31.82	32.05	32.53	32.62	32.65	32.69	32.63
17.00	33.39	33.61	33.86	34.38	34.47	35.41	34.55	34.48
18.00	35.17	35.41	35.68	36.23	36.33	36.37	36.41	36.34
19.00	36.95	37.21	37.50	38.08	38.19	38.23	38.28	38.20
20.00	38.74	39.02	39.32	39.94	40.05	40.10	40.15	40.06
21.00	40.53	40.82	41.15	41.81	41.93	41.98	42.03	41.94
22.00	42.33	42.64	42.98	43.68	43.81	43.86	43.92	43.82
23.00	44.13	44.46	44.82	45.56	45.70	45.75	45.81	45.71
24.00	45.92	46.28	46.66	47.44	47.60	47.65	47.71	47.60
25.00	47.73	48.10	48.51	49.34	49.50	49.56	49.62	49.50
26.00	49.55	49.94	50.37	51.24	51.40	51.47	51.54	51.41
27.00	51.37	51.78	52.23	53.15	53.32	53.39	53.47	53.33
28.00	53.19	53.62	54.09	55.07	55.25	55.32	55.40	55.25
29.00	55.02	55.47	55.97	56.99	57.18	57.26	57.34	57.19
30.00	56.84	57.32	57.85	58.93	59.13	59.21	59.30	59.13
31.00	58.68	59.18	59.73	60.87	61.08	61.16	61.25	61.09
32.00	60.52	61.05	61.63	62.82	63.04	63.13	63.23	63.05
33.00	62.37	62.93	63.53	64.79	65.02	65.11	65.21	65.02
34.00	64.23	64.81	65.44	66.76	67.00	67.10	67.21	67.00
35.00	66.10	66.71	67.36	68.75	59.00	69.11	69.22	69.00
36.00	67.97	68.61	69.30	70.75	71.01	71.12	71.24	71.01
37.00	69.85	70.52	71.24	72.75	73.03	73.14	73.26	73.03
38.00	71.75	72.45	73.19	74.77	75.06	75.18	75.31	75.06
39.00	73.65	74.38	75.16	76.81	77.12	77.24	77.37	77.10
40.00	75.57	76.33	77.14	78.86	79.17	79.30	79.44	79.17
41.00	77.50	78.29	79.13	80.92	81.25	81.38	81.53	81.25
42.00	79.44	80.26	81.14	83.00	83.34	83.48	83.63	83.33
43.00	81.39	82.25	83.17	85.10	85.46	85.60	85.75	85.44
44.00	83.36	84.25	85.20	87.21	87.58	87.72	87.88	87.56
45.00	85.34	86.26	87.26	89.33	89.71	89.86	90.03	89.69
46.00	87.35	88.30	89.34	91.47	91.87	92.03	92.20	91.84

TABLE X. EXPANDED BÉ.–D. E.–D. S

(100°/60°)

D. E.	30.00	42.00	55.00	82.00	87.00	89.00	91.2	90.7
Ash	0.28	0.28	0.30	0.41	0.61	0.61	0.61	1.22
Baumé	Per Cent Dry Substance							
0.00	1.24	1.25	1.26	1.27	1.27	1.27	1.27	1.27
1.00	2.98	3.00	3.03	3.06	3.07	3.07	3.07	3.06
2.00	4.73	4.76	4.80	4.85	4.87	4.87	4.88	4.86
3.00	6.48	6.52	6.57	6.65	6.66	6.67	6.68	6.66
4.00	8.24	8.29	8.35	8.44	8.46	8.47	8.48	8.46
5.00	9.99	10.05	10.13	10.25	10.27	10.28	10.29	10.27
6.00	11.75	11.82	11.91	12.05	12.08	12.09	12.10	12.08
7.00	13.51	13.59	13.70	13.86	13.90	13.91	13.92	13.89
8.00	15.28	15.37	15.49	15.68	15.71	15.73	15.75	15.71
9.00	17.05	17.15	17.28	17.50	17.54	17.56	17.58	17.53
10.00	18.81	18.93	19.07	19.32	19.37	19.39	19.41	19.36
11.00	20.58	20.71	20.87	21.14	21.20	21.22	21.24	21.19
12.00	22.36	22.50	22.67	22.98	23.04	23.06	23.09	23.03
13.00	24.13	24.28	24.48	24.81	24.87	24.90	24.93	24.87
14.00	25.91	26.08	26.29	26.65	26.72	26.75	26.78	26.72
15.00	27.69	27.88	28.10	28.50	28.58	28.61	28.64	28.57
16.00	29.48	29.68	29.92	30.35	30.44	30.47	30.51	30.42
17.00	31.26	31.48	31.74	32.20	32.29	32.33	32.37	32.28
18.00	33.06	33.29	33.56	34.06	34.16	34.20	34.24	34.15
19.00	34.85	35.10	35.39	35.93	36.03	36.07	36.12	36.02
20.00	36.65	36.92	37.22	37.81	37.92	37.96	38.01	37.90
21.00	38.46	38.74	39.06	39.68	39.80	39.85	39.90	39.79
22.00	40.26	40.56	40.91	41.56	41.69	41.74	41.80	41.68
23.00	42.07	42.39	42.76	43.45	43.59	43.64	43.70	43.58
24.00	43.89	44.23	44.61	45.35	45.49	45.55	45.61	45.49
25.00	45.71	46.07	46.47	47.26	47.41	47.47	47.54	47.40
26.00	47.53	47.91	48.34	49.18	49.34	49.40	49.47	49.32
27.00	49.37	49.77	50.22	51.10	51.26	51.33	51.40	51.25
28.00	51.21	51.63	52.10	53.03	53.20	53.27	53.35	53.19
29.00	53.05	53.49	53.99	54.97	55.16	55.23	55.31	55.14
30.00	54.89	55.36	55.88	56.92	57.11	57.19	57.28	57.10
31.00	56.75	57.24	57.79	58.87	59.08	59.16	59.25	59.06
32.00	58.61	59.13	59.70	60.85	61.06	61.15	61.24	61.04
33.00	60.49	61.03	61.62	62.83	63.05	63.14	63.24	63.03
34.00	62.36	62.93	63.55	64.82	65.06	65.15	65.25	65.03
35.00	64.26	64.85	65.49	66.82	67.07	67.17	67.28	67.04
36.00	66.15	66.77	67.44	68.84	69.10	69.20	69.31	69.07
37.00	68.05	68.70	69.40	70.87	71.14	71.25	71.37	71.10
38.00	69.97	70.65	71.37	72.91	73.19	73.30	73.42	73.15
39.00	71.89	72.60	73.36	74.96	75.25	75.37	75.50	75.22
40.00	73.83	74.57	75.36	77.03	77.34	77.46	77.60	77.31
41.00	75.78	76.55	77.38	79.12	79.44	79.57	79.71	79.41
42.00	77.75	78.55	79.41	81.23	81.57	81.70	81.85	81.53
43.00	79.72	80.56	81.46	83.35	83.70	83.84	83.99	83.67
44.00	81.71	82.58	83.53	85.49	85.85	86.00	86.16	85.82
45.00	83.71	84.62	85.61	87.65	88.03	88.18	88.35	87.99
46.00	85.74	86.68	87.70	89.83	90.22	90.38	90.55	90.18
47.00	87.76	88.75	89.82	92.04	92.45	92.61	92.79	92.40

TABLE XI. EXPANDED BÉ.–D. E.–D. S.

(60°/60° F.)

D. E.	30.00	42.00	55.00	82.00	87.00	89.00	91.2	90.7
Ash	0.28	0.28	0.30	0.41	0.61	0.61	0.61	1.22
Baumé	Per Cent Dry Substance							
0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1.00	1.73	1.74	1.74	1.77	1.77	1.77	1.77	1.76
2.00	3.45	3.47	3.48	3.53	3.54	3.54	3.54	3.53
3.00	5.18	5.21	5.23	5.30	5.31	5.31	5.31	5.30
4.00	6.93	6.96	6.98	7.07	7.08	7.09	7.10	7.08
5.00	8.67	8.71	8.74	8.85	8.86	8.87	8.88	8.86
6.00	10.41	10.46	10.50	10.63	10.65	10.66	10.67	10.64
7.00	12.15	12.21	12.26	12.42	12.45	12.46	12.47	12.43
8.00	13.89	13.96	14.03	14.21	14.24	14.25	14.27	14.23
9.00	15.63	15.72	15.80	16.00	16.04	16.05	16.07	16.03
10.00	17.38	17.48	17.58	17.80	17.84	17.86	17.88	17.83
11.00	19.14	19.25	19.36	19.61	19.65	19.67	19.69	19.64
12.00	20.90	21.02	21.14	21.42	21.47	21.49	21.51	21.46
13.00	22.67	22.80	22.93	23.23	23.29	23.31	23.33	23.28
14.00	24.44	24.58	24.72	25.06	25.12	25.14	25.17	25.11
15.00	26.20	26.36	26.52	26.89	26.95	26.98	27.01	26.94
16.00	27.97	28.14	28.32	28.72	28.79	28.82	28.85	28.78
17.00	29.74	29.93	30.13	30.55	30.63	30.66	30.69	30.62
18.00	31.52	31.73	31.94	32.40	32.49	32.52	32.55	32.47
19.00	33.31	33.53	33.76	34.25	34.35	34.38	34.42	34.33
20.00	35.10	35.33	35.58	36.11	36.21	36.25	36.29	36.19
21.00	36.89	37.14	37.41	37.97	38.08	38.12	38.17	38.07
22.00	38.69	38.96	39.25	39.85	39.96	40.00	40.05	39.95
23.00	40.49	40.78	41.09	41.73	41.84	41.89	41.94	41.83
24.00	42.30	42.60	42.94	43.61	43.74	43.79	43.85	43.73
25.00	44.12	44.44	44.79	45.51	45.65	45.70	45.76	45.65
26.00	45.94	46.28	46.65	47.42	47.56	47.62	47.68	47.54
27.00	47.76	48.12	48.52	49.34	49.49	49.55	49.61	49.47
28.00	49.58	49.97	50.39	51.26	51.42	51.48	51.55	51.41
29.00	51.42	51.83	52.28	53.19	53.36	53.43	53.50	53.35
30.00	53.27	53.70	54.17	55.14	55.32	55.39	55.47	55.31
31.00	55.13	55.58	56.07	57.09	57.28	57.36	57.44	57.27
32.00	56.99	57.47	57.98	59.06	59.26	59.34	59.43	59.25
33.00	58.85	59.36	59.90	61.05	61.26	61.34	61.43	61.24
34.00	60.73	61.26	61.83	63.04	63.27	63.35	63.45	63.24
35.00	62.62	63.18	63.77	65.05	65.29	65.38	65.48	65.26
36.00	64.51	65.10	65.73	67.07	67.32	67.42	67.53	67.29
37.00	66.42	67.04	67.70	69.11	69.37	69.47	69.58	69.33
38.00	68.34	68.99	69.67	71.16	71.43	71.54	71.65	71.40
39.00	70.27	70.95	71.67	73.23	73.51	73.62	73.74	73.48
40.00	72.20	72.92	73.68	75.31	75.61	75.73	75.86	75.57
41.00	74.16	74.92	75.71	77.41	77.73	77.85	77.99	77.68
42.00	76.14	76.92	77.76	79.53	79.86	79.99	80.13	79.81
43.00	78.12	78.94	79.83	81.67	82.01	82.15	82.30	81.96
44.00	80.14	80.99	81.91	83.83	84.19	84.33	84.49	84.13
45.00	82.16	83.05	84.01	86.01	86.38	86.53	86.70	86.32
46.00	84.21	85.14	86.14	88.21	88.60	88.75	88.92	88.53
47.00	86.29	87.25	88.28	90.45	90.84	90.99	91.16	90.76
48.00	88.39	89.38	90.45	92.67	93.08	93.24	93.42	93.00

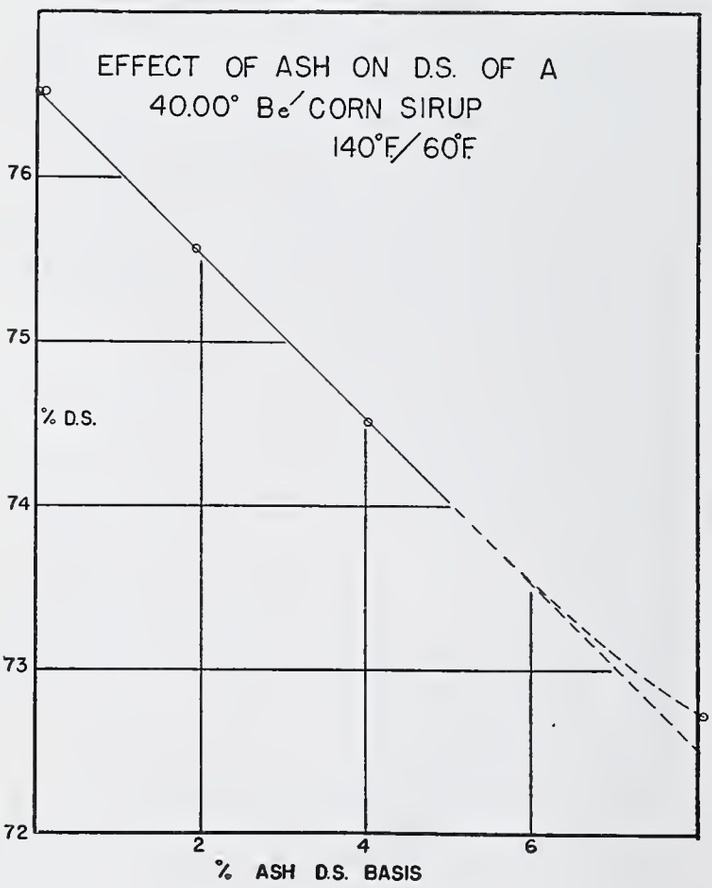

FIGURE 3

TABLE XII. TEMPERATURE CORRECTIONS

(Standard hydrometer, range 10° Bé.)

Observed Bé.	140° to 100° F.	140° to 60° F.	100° to 60° F
	42.00 D. E. C. S. U.		
−2.01	1.30	2.01	
−0.71	1.29	2.02	0.71
0.00	1.29	2.02	0.72
5.00	1.26	2.04	0.77
10.00	1.23	2.06	0.82
15.00	1.19	2.06	0.86
20.00	1.15	2.03	0.88
25.00	1.10	1.99	0.89
30.00	1.04	1.93	0.89
35.00	0.97	1.83	0.87
40.00	0.89	1.70	0.83
45.00	0.79	1.53	0.75
	55.00 D. E. C. S. U.		
−2.01	1.30	2.01	
−0.71	1.29	2.02	0.71
0.00	1.29	2.03	0.72
5.00	1.25	2.06	0.79
10.00	1.22	2.07	0.84
15.00	1.18	2.06	0.87
20.00	1.14	2.04	0.90
25.00	1.09	2.00	0.91
30.00	1.03	1.93	0.90
35.00	0.96	1.83	0.88
40.00	0.88	1.69	0.83
45.00	0.79	1.52	0.75
	89.00 D. E. Sugar		
−2.01	1.30	2.01	
−0.71	1.30	2.03	0.71
0.00	1.29	2.03	0.72
5.00	1.26	2.06	0.79
10.00	1.23	2.08	0.84
15.00	1.18	2.08	0.89
20.00	1.14	2.06	0.91
25.00	1.09	2.01	0.92
30.00	1.03	1.93	0.91
35.00	0.96	1.82	0.88
40.00	0.87	1.68	0.82
45.00	0.77	1.50	0.74

Table XIII. Temperature Corrections

(In Baumé to be added or subtracted to observed Baumé to reduce to 100° F.)

Temperature, ° F.	Observed Baumé 0.0	5	10	15	20	25	30	35	40	45
	Subtract from Observed Baumé									
60	0.72	0.79	0.84	0.87	0.90	0.91	0.90	0.88	0.84	0.77
65	0.64	0.70	0.75	0.77	0.79	0.80	0.79	0.77	0.73	0.66
70	0.56	0.61	0.65	0.67	0.69	0.69	0.68	0.67	0.63	0.57
75	0.48	0.52	0.55	0.57	0.58	0.58	0.57	0.56	0.52	0.48
80	0.39	0.43	0.44	0.46	0.47	0.47	0.46	0.45	0.42	0.39
85	0.30	0.33	0.34	0.35	0.36	0.36	0.35	0.33	0.31	0.29
90	0.21	0.23	0.23	0.24	0.24	0.24	0.24	0.22	0.21	0.19
95	0.11	0.12	0.12	0.12	0.13	0.12	0.12	0.11	0.10	0.09
	Add to Observed Baumé									
105	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.10	0.10
110	0.24	0.24	0.25	0.25	0.25	0.25	0.24	0.23	0.21	0.19
115	0.37	0.38	0.39	0.39	0.39	0.38	0.36	0.35	0.32	0.29
120	0.51	0.52	0.53	0.54	0.53	0.51	0.49	0.46	0.43	0.39
125	0.66	0.68	0.69	0.69	0.67	0.65	0.62	0.58	0.54	0.49
130	0.84	0.84	0.85	0.84	0.82	0.79	0.75	0.71	0.65	0.59
135	1.05	1.04	1.03	1.01	0.98	0.94	0.89	0.83	0.76	0.69
140	1.29	1.26	1.23	1.19	1.14	1.09	1.03	0.96	0.88	0.79

Table XIV. Temperature Corrections

(In Baumé to be added to observed Baumé to reduce to 60° F.)

Temperature, ° F.	Observed Baumé 0.0	5	10	15	20	25	30	35	40	45
60	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
65	0.08	0.09	0.09	0.10	0.11	0.11	0.11	0.11	0.11	0.10
70	0.16	0.18	0.19	0.20	0.21	0.22	0.22	0.21	0.21	0.19
75	0.24	0.27	0.29	0.30	0.32	0.33	0.33	0.32	0.31	0.28
80	0.33	0.36	0.40	0.41	0.43	0.44	0.44	0.43	0.41	0.37
85	0.42	0.46	0.50	0.52	0.54	0.55	0.55	0.55	0.52	0.47
90	0.51	0.56	0.61	0.63	0.66	0.67	0.66	0.66	0.62	0.56
95	0.61	0.67	0.72	0.75	0.77	0.79	0.78	0.77	0.73	0.66
100	0.72	0.79	0.84	0.87	0.90	0.91	0.90	0.88	0.83	0.75
105	0.84	0.91	0.96	0.99	1.02	1.03	1.02	1.00	0.93	0.85
110	0.96	1.03	1.09	1.12	1.15	1.16	1.14	1.11	1.04	0.94
115	1.09	1.17	1.23	1.26	1.29	1.29	1.26	1.23	1.15	1.03
120	1.23	1.31	1.37	1.41	1.43	1.42	1.39	1.34	1.25	1.13
125	1.39	1.47	1.53	1.56	1.57	1.56	1.52	1.46	1.36	1.23
130	1.57	1.64	1.69	1.71	1.72	1.70	1.65	1.59	1.47	1.33
135	1.78	1.84	1.87	1.88	1.88	1.85	1.79	1.71	1.58	1.42
140	2.03	2.06	2.07	2.06	2.04	2.00	1.93	1.83	1.69	1.52

Table XV. Ash Correction for 40.00° Bé. (140°/60° F.)

Ash %	Decrease in Dry Substance %
1.0	0.51
2.0	1.01
3.0	1.51
4.0	2.00
5.0	2.50
6.0	2.98
7.0	3.41
8.0	3.76

The ash content of a sirup affects the dry substance for a given Baumé. Of the four sirups used, the first three have ash contents essentially in proportion to the dextrose equivalent, thus permitting a cross plot wherein coordinates dry substance and dextrose equivalent are plotted for the corresponding values for each Baumé.

In this plot each millimeter equaled 0.10 per cent D. S. and 0.10 D. E. The curve for each Baumé was found to fit a straight line within the experimental error of 0.01° Bé. or 0.02 per cent D. S. This observation was checked by a number of points at 32.8, 36.5, 82.0, and 83.6 D. E. with sirup prepared or obtained for this particular purpose. The data obtained by extrapolation and interpolation for the usual dextrose equivalents at 60.0°, 100.0°, and 140.0° F. are shown in Tables IX, X, and XI.

As noted, the ash content of a given sirup changes the dry substance for a given Baumé—that is, the addition of ash lowers the dry substance for a given Baumé. This ash effect becomes very important in the manufacture of refined sugar, where second and third corn sugar sirups and hydrol are obtained with increasing amounts of ash, up to 8 per cent. Thus a table of ash corrections is most important. In Tables IX, X, and XI, the last two columns are headed D. E. 91.2, ash 0.61 per cent, and D. E. 90.7, ash 1.22 per cent. If these dextrose equivalents are reduced to an ash-free basis they are the same. Thus the difference between these dry substance values for all Baumés becomes a measure of the effect of 0.61 per cent ash. This is 0 at 0° Bé. and increases as the Baumé is increased. Thus it would be possible to prepare a series of such curves for different amounts of ash, provided a cross curve for a given Baumé was obtained—that is, a curve showing the effect of increasing amounts of ash on the dry substance in a given sirup having a constant dextrose equivalent (carbohydrate solids basis) and a constant Baumé.

Table XVI. Commercial Table

(Bé. = Bé. 140° F./60° F. + 1.00°)

Baumé	Specific Gravity[a]	Pounds per Gallon[a]	Dextrose Equivalent and Ash 30.00 0.28	42.00 0.28	55.00 0.30	82.00 0.41	87.00 0.61	89.00 0.61	91.2 0.61	90.7 1.22
			Per Cent Dry Substance							
0.00	1.0000	8.328	1.76	1.77	1.78	1.80	1.80	1.80	1.80	1.80
1.00	1.0069	8.386	3.50	3.52	3.53	3.58	3.59	3.59	3.59	3.59
2.00	1.0140	8.445	5.24	5.27	5.29	5.36	5.38	5.38	5.38	5.37
3.00	1.0211	8.504	6.98	7.02	7.06	7.15	7.17	7.17	7.18	7.17
4.00	1.0285	8.565	8.72	8.77	8.82	8.94	8.96	8.96	8.97	8.96
5.00	1.0358	8.626	10.47	10.53	10.59	10.73	10.76	10.76	10.77	10.76
6.00	1.0433	8.689	12.22	12.29	12.36	12.53	12.56	12.57	12.58	12.56
7.00	1.0508	8.751	13.97	14.05	14.14	14.33	14.36	14.37	14.39	14.37
8.00	1.0585	8.815	15.73	15.82	15.91	16.13	16.17	16.18	16.20	16.17
9.00	1.0663	8.880	17.48	17.58	17.69	17.94	17.98	18.00	18.02	17.99
10.00	1.0742	8.946	19.24	19.35	19.48	19.75	19.79	19.81	19.83	19.80
11.00	1.0822	9.013	21.00	21.13	21.26	21.56	21.61	21.63	21.65	21.62
12.00	1.0903	9.081	22.76	22.90	23.05	23.38	23.44	23.46	23.48	23.45
13.00	1.0986	9.150	24.52	24.68	24.84	25.20	25.26	25.29	25.31	25.27
14.00	1.1071	9.220	26.29	26.46	26.64	27.02	27.09	27.12	27.15	27.11
15.00	1.1156	9.291	28.06	28.24	28.44	28.85	28.93	28.96	28.99	28.94
16.00	1.1242	9.362	29.83	30.03	30.24	30.69	30.77	30.80	30.84	30.78
17.00	1.1330	9.436	31.61	31.82	32.05	32.53	32.62	32.65	32.69	32.63
18.00	1.1419	9.510	33.39	33.61	33.86	34.38	34.47	34.51	34.55	34.48
19.00	1.1510	9.586	35.17	35.41	35.68	36.23	36.33	36.37	36.41	36.34
20.00	1.1602	9.662	36.95	37.21	37.50	38.08	38.19	38.23	38.28	38.20
21.00	1.1696	9.741	38.73	39.01	39.32	39.94	40.05	40.10	40.15	40.06
22.00	1.1791	9.820	40.52	40.82	41.15	41.81	41.93	41.98	42.03	41.94
23.00	1.1888	9.900	42.32	42.63	42.98	43.68	43.81	43.86	43.92	43.82
24.00	1.1986	9.982	44.12	44.45	44.82	45.56	45.70	45.75	45.81	45.71
25.00	1.2086	10.065	45.92	46.27	46.66	47.44	47.60	47.65	47.71	47.60
26.00	1.2188	10.150	47.73	48.10	48.51	49.34	49.50	49.56	49.62	49.50
27.00	1.2291	10.236	49.54	49.93	50.37	51.24	51.40	51.47	51.54	51.41
28.00	1.2396	10.324	51.36	51.77	52.23	53.15	53.32	53.39	53.47	53.33
29.00	1.2503	10.413	53.19	53.62	54.09	55.07	55.25	55.32	55.40	55.25
30.00	1.2612	10.504	55.02	55.47	55.97	56.99	57.18	57.26	57.34	57.19
31.00	1.2723	10.596	56.85	57.33	57.85	58.93	59.13	59.21	59.30	59.13
32.00	1.2836	10.690	58.69	59.19	59.73	60.87	61.08	61.16	61.25	61.09
33.00	1.2950	10.785	60.53	61.06	61.63	62.82	63.04	63.13	63.22	63.05
34.00	1.3067	10.883	61.39	62.94	63.53	64.79	65.02	65.11	65.21	65.02
35.00	1.3186	10.982	64.25	64.83	65.44	66.76	67.01	67.10	67.21	67.00
36.00	1.3307	11.083	66.11	66.72	67.36	68.75	69.01	69.11	69.22	69.00
37.00	1.3430	11.185	67.99	68.62	69.30	70.75	71.01	71.12	71.24	71.01
38.00	1.3556	11.289	69.88	70.54	71.24	72.75	73.03	73.14	73.26	73.03
39.00	1.3684	11.396	71.77	72.46	73.19	74.78	75.07	75.18	75.31	75.06
40.00	1.3815	11.505	73.66	74.39	75.16	76.82	77.12	77.24	77.37	77.10
41.00	1.3947	11.615	75.58	76.34	77.14	78.86	79.18	79.30	79.44	79.17
42.00	1.4083	11.729	77.51	78.30	79.13	80.92	81.25	81.38	81.52	81.25
43.00	1.4221	11.844	79.45	80.27	81.14	83.00	83.35	83.48	83.63	83.33
44.00	1.4361	11.961	81.39	82.25	83.17	85.10	85.46	85.60	85.75	85.44
45.00	1.4506	12.081	83.36	84.25	85.20	87.21	87.58	87.72	87.88	87.56
46.00	1.4652	12.202	85.34	86.26	87.26	89.33	89.71	89.86	90.03	89.69
47.00	1.4802	12.328	87.33	88.29	89.34	91.47	91.87	92.03	92.21	91.84

[a] In air.

The ash encountered in this industry is largely sodium chloride formed from the neutralization of the hydrolyzing acid (hydrochloric) by soda ash. This ash is increased automatically as crystalline dextrose is withdrawn from the massecuite. The method chosen for establishing a cross curve was as follows:

It was assumed that the ash effect would be essentially independent of dextrose equivalent—that is, dependent upon a ratio of carbohydrate to salt. Since corn sirup is more easily dried than sugar and free from the criticism of instability on drying, the test was made with corn sirup. A 5-gallon conversion was made in pilot plant equipment to approximately 42 D. E., using sulfuric acid for hydrolysis and barium carbonate for neutralization. This was refined in the usual manner, and concentrated to 40° Bé. at 140° F. (41° Bé. commercial). This sirup was substantially ash-free (0.07 per cent). To this was added sodium chloride in amounts to make 1.00, 2.00, 4.00 and 8.00 per cent (dry substance basis). Sirups were made above and below 40° Bé. (140°/60° F.) and specific gravity and dry substances were obtained. Since the Baumé range was small, a straight line was drawn between the two points, the values being plotted on a scale which was five times that previously used, and the exact values for 40.00° Bé. (140°/60° F.) were obtained for each of the salt concentrations.

The data obtained together with the calculated ash-free point appear in Figure 3. The specific values are shown in Table XV.

The curve obtained is a straight line up to 6 per cent and bends slightly thereafter. For commercial work the salt correction may be taken as a straight line. Thus a cross curve for salt effect has been obtained for a heavy Baumé, and the data, together with the data for salt effect for 0.61 per cent ash for all Baumés, enable a family of curves to be drawn of high precision and of greater accuracy than is generally required for factory work.

Attention was called above to the commercial method of determining Baumé—namely, the reading at 140° F. plus 1.00° Bé. or Bé. = Bé. 140°/60° F. + 1.00. All sirups are sold in the industry on this basis. Table XVI is the commercial table that has been accepted by the Corn Industries Research Foundation for its member companies for use in the sale of its products. The specific gravities and the weights per gallon are those in air. This method of assignment was suggested by the National Bureau of Standards for use in the preparation of a commercial table.

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Pressure Stopcock

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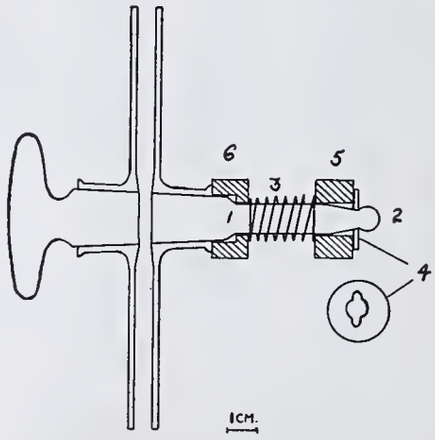
With present laboratory techniques it is frequently necessary to employ for gas absorption, gas analysis, solvent extraction, and the like, glass systems which operate under superatmospheric pressures. As far as this writer is aware only special, commercially manufactured pressure stopcocks operate reliably. Rubber bands and metal clips either impede the operation of the stopcock or do not prevent lifting of the plug, thereby causing escape of the confined material.

The design of the commercially available stopcock requires special glass-drilling equipment and is, therefore, not well adapted to duplication at the laboratory. A pressure stopcock which can be adapted from the generally stocked glass stopcock, with ordinary glass-blowing skill and at insignificant cost should, therefore, prove of help to the laboratory worker.

The pressure stopcock presented here has been designed to fill this gap. Depending upon spring strength and the type of stopcock lubricant pressures of 30 pounds or more may be applied. The stopcock has proved its worth in various apparatus.

The drawing illustrates a standard 4 mm. Pyrex stopcock. It is recommended that stopcocks made from Pyrex be used in the assembly of the pressure stopcock. Only those possessing a protruding portion of the barrel for a rubber ring should be selected. As an added precaution against damage, during sealing, the ground portion of the barrel may be protected by a moist strip of asbestos paper. Stopcocks made from soft glass could probably be used only if reground after the sealing operation.

1 is a solid glass rod of slightly smaller diameter than that of the plug body, sealed to the latter without undue heating of the ground portion of the plug. This seal should be carefully annealed. The rod is drawn out in the shape indicated by 2 and the rounded portion is flattened with forceps or pliers to fit through the elongated portion of the hole in the washer. Part 6 is machined from a metal bar of suitable size or a nut may be adapted. It rests against the evenly ground reinforced rim of the stopcock shell and fits rather loosely over the extension of the stopcock plug. 5 designates a metal bar with a hole of about the same diameter as part 6. Spring 3 should be of suitable strength and its ends should preferably be squared to ensure proper seating against 5 and 6.



The stopcock is assembled by inserting the properly lubricated plug (*1*) into the stopcock shell, slipping cap 6, spring 3, and part 5 over the plug extension, and securing these parts by locking plate 4, which is turned 90 degrees after insertion.

Exact dimensions depend on the stopcock size applied, but the proportions of the various component parts are apparent from the drawing.

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Analysis of the Thixotropy of Pigment-Vehicle Suspensions

Basic Principles of the Hysteresis Loop

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THIXOTROPIC systems have been studied in numerous ways, but an investigation of the subject by means of the hysteresis loop—one of the most fruitful methods—seems to have been neglected. Very few references to the loop occur in the literature. An early paper by Hatschek (*9*, 1913) shows data that if replotted would give the loop. Ostwald (*11*), much later, observed thixotropic hysteresis behavior and would have obtained the characteristic loop if he had plotted his data in the necessary manner. Reiner (*14*) lists non-Newtonian types giving hysteresis loops. Pryce-Jones (*12*) shows a graph of a hysteresis loop and at the same time states a cardinal rheological principle: Thixotropy cannot be determined from a single consistency curve. The importance of the Pryce-Jones principle has not been universally recognized by rheologists.

To learn something of the extent and nature of thixotropic change in breakdown or buildup, it is necessary to have data that cover both the before and after states. This requires at least two curves. The hysteresis loop, obtained by means of the rotational viscometer (described by one of the authors in a recent paper, *6*) is the graph of both states. The loop is obtained by plotting both curves together—i. e., the upcurve, demonstrating breakdown, and the downcurve, showing the particular state of equilibrium to which the material has been brought. Instead of the loop, two downcurves obtained at two different thixotropic levels can be employed. In either case two curves are always necessary.

The Criterion of Thixotropy

A true sol has been defined as a colloidal suspension that has zero yield value. Freundlich (*3*) describes a thixotropic material as one that will undergo isothermally a gel/sol/gel transformation, but is very careful to qualify this definition by pointing out that the conversion to a complete sol is "a specially characteristic and limiting case of thixotropy". This qualification permits such suspensions as paints and printing inks to be classified as thixotropic. Freundlich further adds that suspensions not truly colloidal but containing particles of microscopic size such as "pastes of clay, etc.", "show extremely pronounced thixotropic behavior". He also includes under the phenomenon of thixotropy—and this is important—materials like gelatin, which "become less viscous and elastic on shaking or stirring but assume the original values of viscosity and elasticity when left to rest". Since elasticity is associated with the presence of yield value, it is evident that a material that only becomes "less elastic" (in addition to a decrease in viscosity) might still possess yield value. In other words, a complete transformation from a gel to a true sol is not a prerequisite for thixotropy. A partial transformation is all that is necessary. This is the attitude taken by Goodeve (*4*) and it is the one that will be used here.

Controversial Issues

There are four controversial points involved in the rheology of thixotropic systems. It is necessary that the authors' position in regard to them be made clear.

1. THE NONLINEARITY OF THE LOWER END OF THE CONSISTENCY CURVE. One of the primary causes of this curvature has been shown to arise from the gradual change of plug to laminar flow (*7*, *8*). Buckingham (*2*) showed that this condition occurs in capillary tube viscometers because the shearing force decreases from the capillary wall inward. Reiner (*13*) proved that this same condition exists in rotational viscometers and furthermore indicated that this curvature must become shorter when the distance between the bob and cup is decreased. Laboratory experiments have confirmed this conclusion. Hence the curvature, so far as actually known at present, could be entirely due to an instrumental effect and does not necessarily have to arise from any change in the structure of the material under low shearing stresses. If the distance between the bob and cup were infinitesimally small, there probably would be no curvature under such a condition. The authors realize, however, that Reiner's deductions are based on an entirely stable system and that thixotropy and pseudoplasticity might easily increase the length of the nonlinear portion of the curve beyond the amount predicted by Reiner.

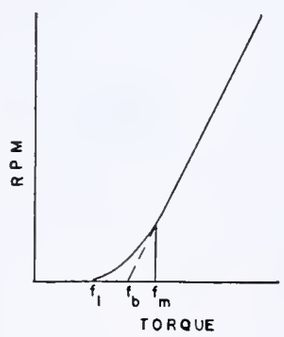

FIGURE 1

2. THE THREE DIFFERENT YIELD VALUES. Houwink (*10*) defines three types of yield values (Figure 1). The lower yield value is the intercept f_l where the first evidence of flow occurs. This is known to be where the outer layer of the "plug" begins to shear (*2*, *7*, *8*). The maximum yield value, f_m, is where the curve becomes linear (*7*, *13*). This is where plug flow is completely gone and the entire flow is laminar. Buckingham (*2*) shows that for capillary viscometers this state is attained only at infinite rates of shear and f_m is the intercept of the asymptote. The intercept, f_B, is the Bingham yield value. The authors' viewpoint is that these three intercepts are not three different yield values but three different functions of one and the same yield value. Reiner (*13*) has shown for rotational viscometers, where thixotropy and pseudoplasticity are not involved, that

$$f_l = 2\pi R_b^2 hf \quad (1)$$

$$f_m = 2\pi R_c^2 hf \quad (2)$$

$$f_B = [4\pi hf \ln (R_c/R_b)]/[1/R_b^2 - 1/R_c^2] \quad (3)$$

where R_c = radius of the cup
R_b = radius of the bob
h = depth of immersion of the bob
f = the real yield value

3. THE APPARENT VISCOSITY. This is a variable quantity depending on the rate of shear. It is obtained by dividing the ordinate (rate of flow; r. p. m.) by the abscissa (force; torque) and multiplying the product by an instrumental constant. It is obvious that apparent viscosity is not the coefficient of viscosity, which by definition is the number of

dynes per sq. cm. that will give a velocity gradient of 1 cm. per second. Other than being the ratio of force to rate of flow it is not "apparent" what it is; hence, it is something of a misnomer and perhaps an unfortunate one.

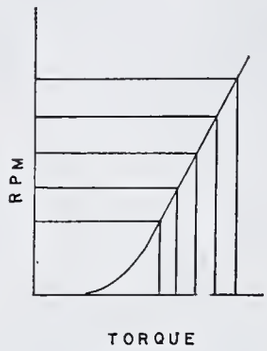

FIGURE 2

An inspection of Figure 2 will show that the ratio of torque—to r. p. m. must decrease with increasing rate of shear—i. e., the apparent viscosity decreases as the speed of the agitation or stirring increases. Unfortunately this reaction is an isothermal one and reversible; hence, any investigator who considers apparent viscosity to be a substitute for coefficient of viscosity will erroneously classify the material giving the consistency curve in Figure 2 as thixotropic. Now all that can be said about the curve is that except for its lower end it is linear (indicating stability) and does not pass through the origin. It does not pass through the origin because the material is plastic—i. e., it has a yield value. The authors' opinion is that in the case of a curve like the one in Figure 2, the apparent viscosity decreases with an increase of rate of shear simply because it is mathematically impossible for it to do otherwise—that is, it is a question of geometry and not one of thixotropic breakdown of the sample during testing.

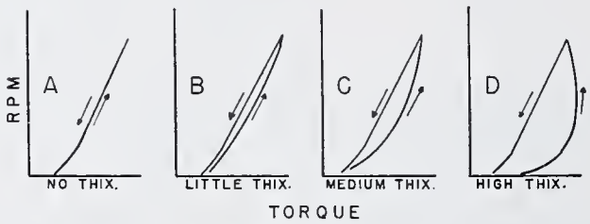

FIGURE 3. THIXOTROPY

Time of measurement constant

4. PLASTIC FLOW WITHOUT LIQUEFACTION. There is a widespread belief that certain suspensions, like finely divided quartz in carbon tetrachloride, are highly thixotropic. If such a suspension, of the right consistency, is put into a test tube and inverted it will not flow out because of its high yield value. If the thumb is placed over the end of the tube and the tube is shaken, it can be observed that the material flows. If the thumb is suddenly removed immediately after shaking has ceased, the material will not flow out. The customary argument is that the material would not flow during shaking if it did not first liquefy—i. e., turn into a sol. When a metal, many degrees below its melting point, is drawn through a die the metal is easily visualized as undergoing plastic flow. In other words, it is a solid before it enters the die, while it passes through the die, and after it is drawn from the die. At no time is the metal considered to be liquid because at no time is it above its melting point. If consistency curves could be obtained for metals like steel, bronze, copper, etc., it seems obvious that they would not be Newtonian. They would all possess intercepts, such as that shown in Figure 3, *A;* yet no one has classified these metals as thixotropic. However, when soft mushy materials of the quartz–carbon tetrachloride suspension type are considered, it is often assumed that flow takes place only when preceded by an intermediate state of liquefaction.

This is an important point, for if it should be true—and the authors believe it is not—then the quartz–carbon tetrachloride type of suspension would be thixotropic in spite of the fact that it gives no hysteresis loop. If such a non-loop-forming material (see also Figure 12) must be accepted as highly thixotropic, a conflict is introduced in our reasoning, for it would mean that at both ends of our series of loops (Figure 3) a high state of thixotropy would exist. In other words, no hysteresis loop *A* and a large hysteresis loop *D* would both indicate a high state of thixotropy. Any investigator examining a series of thixotropic materials like those represented in Figure 3 would unhesitatingly say that *D* was more thixotropic than *C*, and *C* more thixotropic than *B*. It therefore seems unreasonable that there should be a sudden increase in thixotropy in going from *B* to *A*. Consequently the authors have adopted the viewpoint that curve *A* indicates plasticity with no thixotropy. By so doing confusion is eliminated and a large part of the theory of thixotropy becomes rationalized.

The Hysteresis Loop

The method of making the loop has been described in detail in a previous paper (*6*).

Briefly, it is obtained with a rotational viscometer of special design. This instrument is operated through a Graham continuously variable transmission (Briggs and Stratton Mfg. Co.) which enables the investigator to change the r. p. m. without stopping the rotation of the cup. The procedure commences with the upcurve, starting at the lowest practical r. p. m. The speed is increased continuously and rapidly while noting the change in torque induced. At some specified upper limit the speed is reversed and the downcurve taken. If the material is thixotropic, the up- and downcurves when plotted together will not coincide, thus forming the loop (Figure 3, *B*, *C*, *D*).

THE TIME ELEMENT IN THIXOTROPIC BREAKDOWN. If restraints could be placed on the thixotropic material at the beginning of the upcurve so that it could not break down, the consistency curve would follow a straight path, such as T_2A (Figure 4). If the restraints should then be removed and the r. p. m. at *B* maintained, the torque would decrease from T_A until a state of equilibrium is reached at some point T_{An}. Since the product of the two coordinates (r. p. m. × torque) gives a quantity with the dimensions of power, it is evident that the area of the parallelogram OT_AAB must be the minimum power that will break down the material from the thixotropic level T_2A to the thixotropic level T_2A_n using the velocity gradient given at *B*. In actual practice restraints cannot be employed and so the material continuously breaks down as the upcurve is taken. The real upcurve follows a path such as T_2CA_1. If on reaching A_1 the r. p. m. is kept constant and sufficient time is allowed for equilibrium to be attained, the torque will drop from T_{A1} to T_{An}. The downcurve A_nT_2 will be linear, if not too much time is consumed in running it.

As the time taken for the upcurve is increased, the curve moves in the direction of the curve T_2DA_n. If equilibrium points are run—i. e., if sufficient time is allowed at each successive r. p. m. for the thixotropic buildup to equal the thixotropic breakdown—then the upcurve will follow the path T_2DA_n and not the straight path T_2A_n. This means that after equilibrium has been attained

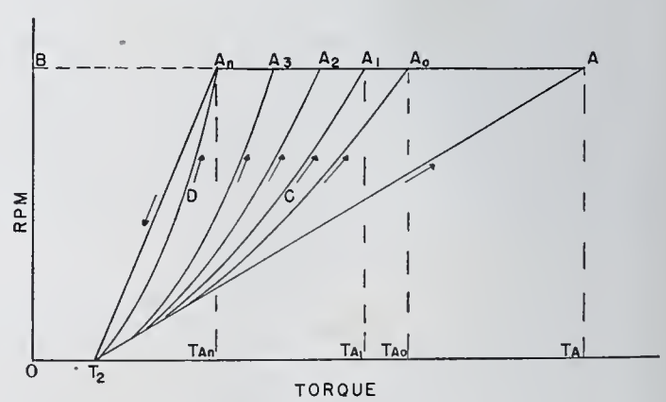

FIGURE 4. EFFECT OF TIME

for any given r. p. m. further breakdown can be had only by increasing the velocity gradient—i. e., by using a higher r. p. m. (more power).

There are two methods then for breaking down thixotropic structure: by increasing the velocity gradient, and by prolonging the time of the applied force. When the time used in taking the measurements for the upcurve is decreased—i. e., when the time during which each successive r. p. m. is allowed to operate is lessened—then the upcurve is displaced in the direction of the curve T_2A. A hypothetical curve run in such a short time that breakdown resulting from the time element is negligible would occupy some position as T_2A_0, where point A_0 would fall between the theoretical point A and the last (fastest) experimentally determinable point, A_1. The curve T_2A_0 should not coincide with curve T_2A because the former is run without restraints while the latter is run with them.

Any curvature of T_2A_0 must result from thixotropic breakdown; but this breakdown has taken place simultaneously with the commencement of flow. In other words the curvature of T_2A_0 arises only from velocity gradient, because the time element is in this case negligible. Such a model of flow would necessitate exceedingly short nondistensible bonds.

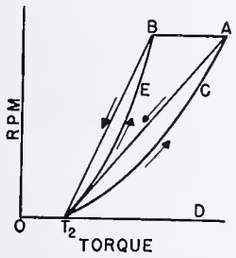

FIGURE 5

When the downcurve is commenced as soon as the maximum r. p. m. is reached, the loop will be referred to simply as a hysteresis loop—e. g., Figure 5, loop T_2CAT_2. When the maximum r. p. m. is applied over a time interval sufficient to attain a state of equilibrium, the loop will be called an equilibrium hysteresis loop—e. g., Figure 5, loop T_2CABT_2. When every point on the upcurve is an equilibrium point, the loop will be called the minimum equilibrium hysteresis loop—e. g., Figure 5, loop T_2EBT_2. The consistency of the material at point A is given by its plastic viscosity, derived from the angle DT_2A; its yield value is obtained from the torque T_2. When the material is broken down further by the prolonged application of the maximum r. p. m. until equilibrium is reached at B, it will have a plastic viscosity derived from the angle DT_2B, and a yield value again obtained from torque T_2. The equations for calculating these consistency factors are derived from Reiner's work (*6, 13*):

$$\text{Plastic viscosity, } U = (T - T_2)\, S/\omega \tag{4}$$

$$\text{Yield value, } f = T_2C \tag{5}$$

$$S = (1/R_b^2 - 1/R_c^2)/4\pi h \tag{6}$$

$$C = S/\ln(R_c/R_b) \tag{7}$$

where

ω = angular velocity

Unlike "apparent viscosity", plastic viscosity has a definite meaning. It is the number of dynes per sq. cm. in excess of the yield value that will produce a velocity gradient of 1 cm. per second. Yield value is the dynes per sq. cm. that will just cause flow. These quantities are given by Equations 4 and 5.

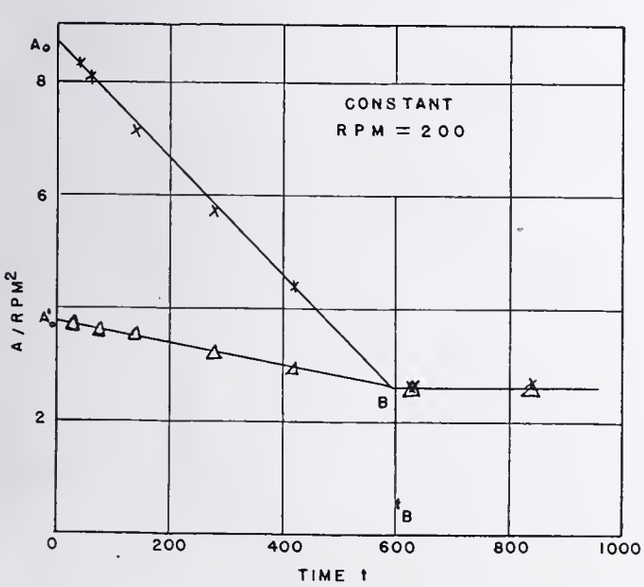

FIGURE 6. EFFECT OF TIME

TABLE I. EXPERIMENTAL DATA FOR FIGURE 6, GIVING EQUATION 8

A = area of hysteresis loop in (gram-cm.) r. p. m.
$A_{eq.}$ = area of equilibrium hysteresis loop. Its dimensions are those of A.
t = time of upcurve in seconds
RPM is constant = 200.

t	$A/(RPM)^2$	$A_{eq.}/(RPM)^2$
31.5	3.7	...
44	...	8.3
61	...	8.1
75	3.6	...
140	3.5	7.1
280	3.2	5.7
420	2.9	4.4
630	2.6	2.6
840	2.6	2.7
1570	...	2.6
2520	2.6	2.6

If experimentally determined areas like the diagrammatic ones in Figure 4, $T_2A_1A_nT_2$, etc., are plotted against their respective times (in seconds), a linear relationship results. This relationship is obtained whether hysteresis loops or equilibrium hysteresis loops are used. Data of this type are plotted in Figure 6, but $A/(RPM)^2$ is used instead of area. The hysteresis loops are given in curve $A_0'B$ and the equilibrium hysteresis loops in curve A_0B. The general formula for these curves is

$$A = A_0 - Nt \; (t \leqq t_B) \tag{8}$$

where N is the proportionality constant, t is the time taken in making the upcurve, and A_0 is the intercept at zero time. The extrapolated point A_0 times its top (r. p. m.)^2 should correspond to the area of the hypothetical equilibrium loop $T_2A_0A_nT_2$ given diagrammatically in Figure 4. Similarly the extrapolated point A_0' times its top (r. p. m.)^2 should correspond to the area of its hypothetical hysteresis loop.

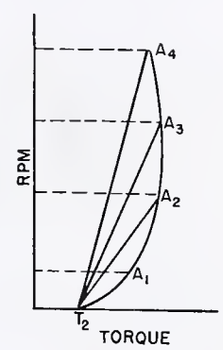

FIGURE 7

TABLE II. EXPERIMENTAL PROOF OF EQUATION 9

		Experimentally Determined Loop Area, A	$(RPM)^2$	$Q = A/(RPM)$
Yellow ink	1	1.61×10^4	1×10^4	1.61
		6.40	4	1.60
		10.0	9	1.11
		26.6	16	1.66
	2	2.64	1	2.64
		10.25	4	2.56
		26.06	9	2.78
		44.17	16	2.76
Red ink	1	1.5	1	1.50
		5.0	4	1.25
		12.9	9	1.43
		22.4	16	1.40
	2	3.63	1	3.63
		14.3	4	3.58
		32.0	9	3.55
		55.0	16	3.44
Blue ink		0.41	1	0.41
		1.82	4	0.45
		3.46	9	0.38
		7.6	16	0.47
White ink		0.54	1	0.54
		2.15	4	0.54
		5.00	9	0.55

THE VELOCITY GRADIENT ELEMENT IN THIXOTROPIC BREAKDOWN. If the time of application of each r. p. m. is maintained constant and the top or maximum r. p. m. is varied, a series of hysteresis loops such as those shown in Figure 7 occurs. The area of these loops plotted against the square of their respective top r. p. m. gives the linear equation

$$(\text{Area})\; A = Q(RPM)^2 \tag{9}$$

where Q is the proportionality constant and (RPM) is the top r. p. m. Equations 8 and 9 constitute the two basic principles involved in the subject of thixotropy. The existence of both principles—breakdown by time, and breakdown by velocity gradient—has been recognized by most investigators of thixot-

ropy; but they probably have not been expressed previously in the form of the equations given here.

The Upcurve

THIXOTROPIC BREAKDOWN. Let a be any point above T_2 on the upcurve (Figure 8). At point a the material has a viscosity given by the cotangent of TT_2a. If upon increasing ω to $\omega + d\omega$ there is no further thixotropic breakdown, the torque would increase from T to T_3. There is breakdown, however, and the torque is increased from T only to T_1; hence, the loss in torque resulting from breakdown is $(T_3 - T_1)$ or bc.

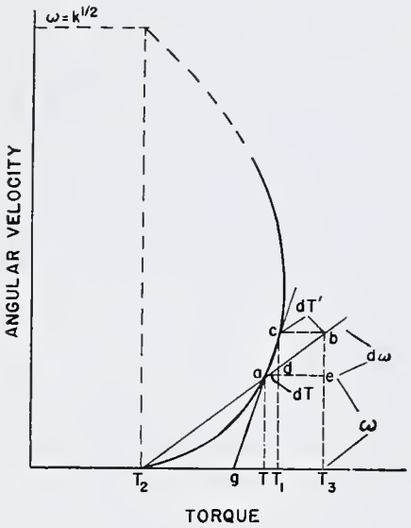

FIGURE 8

Let the total thixotropic loss in torque acquired in going from $\omega = 0$ to $\omega = \omega$ be equal to some quantity in excess of the yield value, such as $(T' - T_2)$. Also let it be assumed that $(T' - T_2)$ is proportional to the velocity gradient. Then

$$T' - T_2 = Z\omega/2\pi R^2hS \quad (10)$$

and

$$dT'/d\omega = Z/2\pi R^2hS \quad (11)$$

where Z is the proportionality constant, $\omega/2\pi R^2hS$ the velocity gradient at radius R, and dT' is the loss of torque resulting from breakdown when ω is increased by the amount $d\omega$. However, bc is also equal to this loss: hence $bc = dT'$.

The triangles abc and aT_2g are similar; consequently,

$$dT'/d\omega = (g - T_2)/\omega \quad (12)$$

From Equation 11 it follows that

$$g - T_2 = Z\,\omega/2\,\pi\,R^2hS \quad (13)$$

From Equations 10 and 13 it follows that

$$T' = g$$

and that the loss in torque from thixotropic breakdown, when ω is increased from $\omega = 0$ to $\omega = \omega$, is equal to $(g - T_2)$. In other words, the tangent at point a gives an intercept g on the torque axis from which the total breakdown (in loss of torque) up to point a can be determined.

EQUATION OF THE UPCURVE. In Figure 8 it can be seen that the net change in torque for an increase in angular velocity of $d\omega$ is ad. Then from the similar triangles bae and aT_2T,

$$dT'/d\omega + dT/d\omega = (T - T_2)/\omega \quad (14)$$

Substituting from Equations 12 and 13 and making the constant $Z/2\pi\,R^2h$ equal to $2/m$ gives

$$dT/d\omega - T/\omega + T_2/\omega = -2/mS \quad (15)$$

TABLE III. PRINTING INK DATA FOR FIGURE 10

RPM	Upcurve Torque	Downcurve Torque	Equilibrium Downcurve Torque
11	114 × 10⁴	49 × 10⁴	37 × 10⁴
14	136	66	45
21	161	88	67
26	182	106	76
33	196	120	87
42	205	133	98
48	216	151	111
55	225	160	121
62	233	174	128
69	236	193	139
76	244	202	150
83	248	214	162
90	253	230	168
97	262	240	180

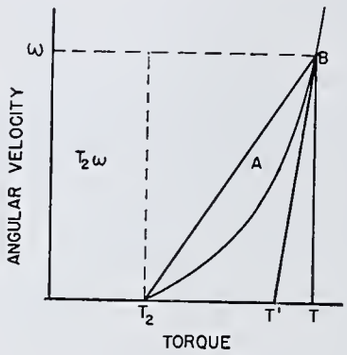

FIGURE 9

Multiplying by $\omega d\omega/\omega^2$ gives

$$\omega dT/\omega^2 - Td\omega/\omega^2 + T_2d\omega/\omega^2 = -2d\omega/mS \quad (16)$$

Then

$$d\,[(T - T_2)S/\omega] = -2d\omega/m\omega \quad (17)$$

or

$$dU = -2d\omega/m\omega \quad (18)$$

Integrating and multiplying by $m/2$

$$mU/2 = -\ln\omega + \text{const.} \quad (19)$$

When U theoretically $= 0$, let ω have the value $k^{1/2}$ (see Figure 8). Then

$$\text{Const.} = \ln k^{1/2} \quad (20)$$

Hence

$$mU/2 = \ln(k^{1/2}/\omega) \quad (21)$$

or

$$e^{mU/2} = k^{1/2}/\omega \quad (22)$$

Squaring puts Equation 22 into a more useful form, as will appear later.

$$e^{mU} = k/\omega^2 \quad (23)$$

Upon substituting $(T - T_2)S/\omega$ for U, the equation of the upcurve is obtained.

$$T = \omega \ln k/mS - 2\omega \ln \omega/mS + T_2 \quad (24)$$

From Equation 23

$$mU = \ln k + \ln(1/\omega^2) \quad (25)$$

Hence, if experimentally determined U is plotted against experimentally determined $(1/\omega^2)$ on semilogarithmic paper a linear relationship should result. This is what happens, as shown in the section on experimental results. Equations 23 and 24 are thus confirmed.

AREA OF THE LOOP. The area, A, of the loop (Figure 9) is

$$A = \int_0^\omega Td\omega - T_2\omega - (T - T_2)\omega/2 \quad (26)$$

Substituting the value of T given in Equation 24 and integrating gives

$$A = \omega^2 \ln k/2mS - \omega^2 \ln \omega/mS + \omega^2/2mS - (T - T_2)\omega/2 \quad (27)$$

From Equation 24

$$\omega^2 \ln k/2mS = \omega^2 \ln \omega/mS + (T - T_2)\omega/2 \quad (28)$$

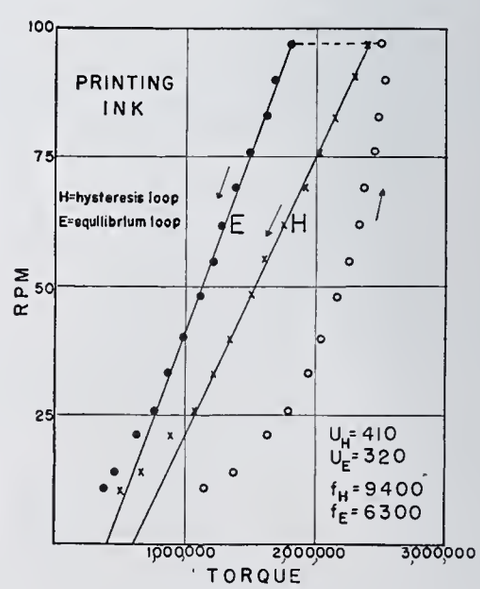

FIGURE 10

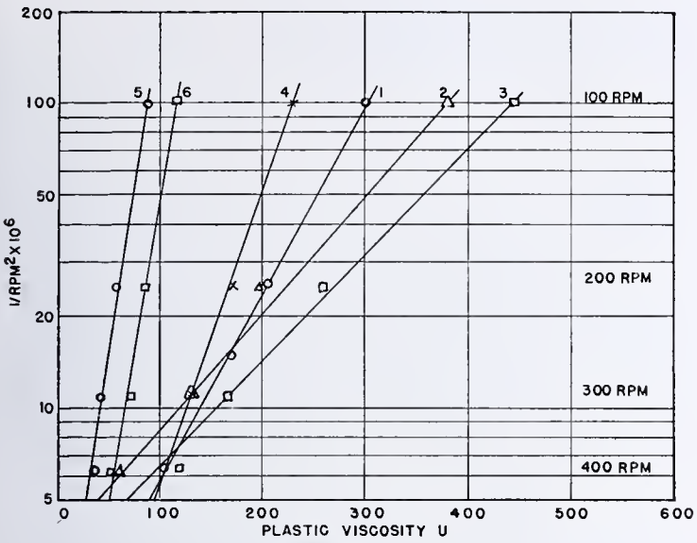

FIGURE 11

Substituting in Equation 27 gives

$$A = \omega^2/2mS \tag{29}$$

which shows that A is directly proportional to ω^2. This is confirmed by the empirical relationship given in Equation 9.

Other important relationships derived from the preceding equations are

$$A = Z\omega^2/8\pi R^2hS \tag{30}$$

$$dT'/d\omega = 4A/\omega^2 \tag{31}$$

$$A = {}^1/_2 \text{ area } \Delta\, T'T_2B \text{ (Figure 9)} \tag{32}$$

$$e^{mU} = k/2mSA \tag{33}$$

$$2/m = M = Z/2\pi R^2h = 2(U_1 - U_2)/\ln(\omega_2^2/\omega_1^2) \tag{34}$$

The proportionality constant, Z, is the loss in torque per unit increase in velocity gradient for the arbitrarily selected time employed in making the upcurve. Z depends on the instrument used in so far as the torque depends on the instrumental dimensions. However, m is independent of the instrument. Therefore $2/m$, or M, will be called "the coefficient of thixotropic breakdown". It is defined as the loss in shearing force per unit area per unit increase in velocity gradient for the arbitrarily selected time employed in making the upcurve.

Experimental Results

In a previous paper (*6*) a number of experimental flow curves were given showing loops of various sizes; consequently, in order to avoid unnecessary repetition only one experimental hysteresis loop is presented here (Figure 10 and Table III). Most pigment suspensions of the printing ink type give loops of this kind. The loop area is best obtained with a planimeter. This area must be multiplied by the proper conversion factors, so that the dimensions are correct for substituting in the various equations given here.

It was found empirically that a linear relationship is obtained by plotting plastic viscosity U against ln $(1/RPM^2)$ when the "time" is maintained constant (Figure 11, Table IV). By "time" is meant the time interval during which the shearing force is allowed to operate for each experimental point on the curve. This interval is regulated and controlled by the operator, who measures a predetermined number of points per minute. Each succeeding point is separated by exactly the same number of r. p. m. The results of the above work lead to the following empirical equation.

$$e^{mU} = \frac{\text{const.}}{RPM^2} \tag{35}$$

This checks the theoretical Equation 23, which was derived on the assumption that when the "time" is maintained constant the loss in torque resulting from thixotropic breakdown is directly proportional to the velocity gradient. Since the empirical and theoretical equations check, this assumption is probably correct.

Goodeve and Whitfield (*5*) use a similar hypothesis. They state that "the rate of breakdown is proportional to the shear". They develop and use this conception, however, in a different manner from that employed in this paper.

Equation 8 is empirical. No theoretical equation has been derived so far that corresponds to it. In this equation the top r. p. m. is maintained constant while the time is changed for each different curve (see Figure 6). In Equation 9 the opposite plan is followed. Time, here, is constant while the top r. p. m. is varied (see Table II). This equation

TABLE IV. EXPERIMENTAL DATA FOR FIGURE 11

[$RPM = 9.55\,\omega$, $Z = 4\pi R^2h/m$, $R = (R_c + R_b)/2$]

	RPM	$1/(RPM)^2$	Plastic Viscosity, U	Z
Yellow ink	100	100×10^{-6}	300	1.07×10^4
	200	25	205	
	260	15	172	
	400	6.3	104	
Red ink 1	100	100	380	1.42
	200	25	198	
	300	11	132	
	400	6.3	60	
2	100	100	447	1.80
	200	25	261	
	300	11	166	
	400	6.3	119	
3	100	100	255	0.74
	200	25	173	
	300	11	132	
	400	6.3	100	
Blue ink	100	100	82	0.21
	200	25	57	
	300	11	44	
	400	6.3	38	
White ink	100	100	115	0.27
	200	25	88	
	300	11	72.5	
	400	6.3	53.5	

TABLE V. QUARTZ IN NUJOL DATA FOR FIGURE 12

RPM	Upcurve Torque	Downcurve Torque
9	161×10^3	145×10^3
13	179	163
20	196	196
27	214	216
34	232	232
40.5	245	247
49	259	261
55	273	273
62	283	288
70	294	296
77	304	308
84	314	319
92	325	332
98	337	340

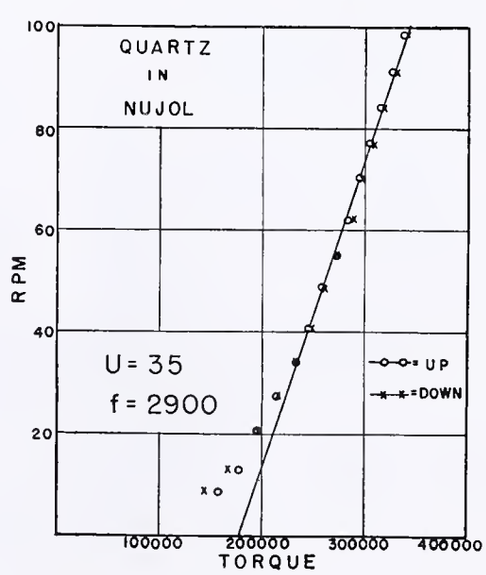

FIGURE 12

Table VI. Carbon Black in Glycerol Data for Figure 13

RPM	Upcurve Torque	Downcurve Torque
9	...	198 × 10³
13	...	210
20	238 × 10³	226
26	235	232
33	238	241
41	244	244
48	254	254
55	260	256
62	275	260
69	275	269
76	275	272
83	275	278
90	278	281
97	281	284
104	284	286
116	286	290
131	299	305
144	308	308
158	314	318
177	324	324
196	336	339

is empirical but it corresponds to the theoretically derived Equation 29.

In deriving Equation 17, m is treated as a constant. Equations 29 and 30 give the relation between A and ω for any single substance, m (or Z) being a constant for a given material. For different substances m (or Z) varies and the relationship between m (or Z) and A is expressed by Equation 33.

It is difficult to find pigment suspensions that give no loops. They can be made, however, and three of them are shown in Figures 12, 13, and 14 (Tables V, VI, and VII). The microscopic glass spheres used in Figure 14 were made in the Interchemical Research Laboratories by a method similar to the one developed by Bloomquest (*1*).

From the authors' viewpoint these non-loop-forming materials are plastic without being thixotropic. The more general conception is that such materials are so highly thixotropic that no apparatus built so far is quick enough to record either the breakdown or the buildup. Obviously, then, this breakdown or buildup is entirely conjectural, for its existence has not been proved experimentally. If conjecture is acceptable, it seems far more reasonable to say that the absence of a loop indicates the absence of thixotropy.

Conclusion

The authors have treated the subject here only in its elemental form—i. e., where the primary change occurring upon agitating a thixotropic suspension is a decrease in plastic viscosity. This treatment is sufficient for many cases. In a later paper an analysis will be given of materials involving a double change—i. e., in both plastic viscosity and yield value.

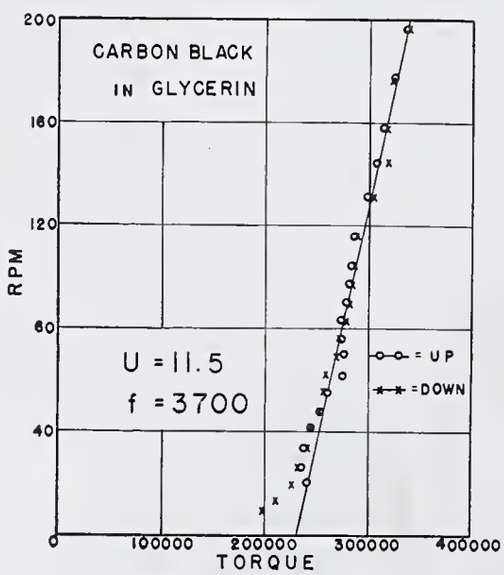

Figure 13

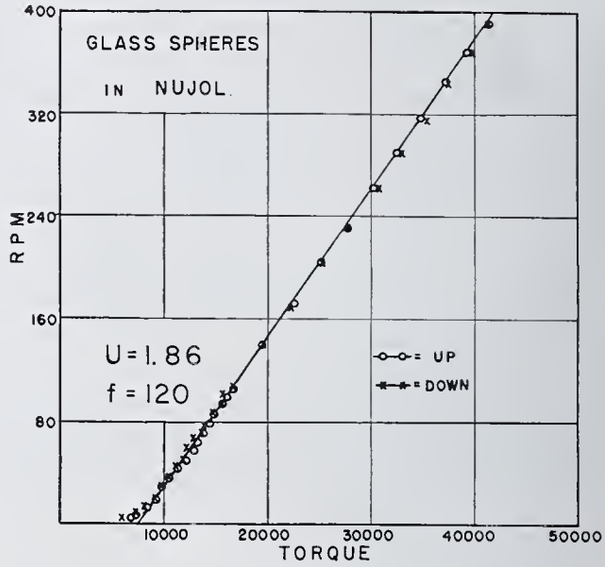

Figure 14

Table VII. Glass Spheres in Nujol Data for Figure 14

RPM	Upcurve Torque	Downcurve Torque
5	67.7 × 10²	59.8 × 10²
8	70.2	72.8
15	83.2	80.5
21	91.0	91.0
30	96.2	98.8
37	104	104
45	112	110
51	120	117
59	128	122
65	132	128
72	138	135
79	143	140
87	148	146
94	156	156
101	161	156
107	166	166
141	195	195
174	226	223
204	252	252
234	278	278
264	301	307
291	325	330
319	349	354
345	372	375
369	393	398
392	414	414

Acknowledgment

The authors are grateful to the Interchemical Corporation for permission to publish and are indebted to Evelyn Berezin for assistance in carrying out the laboratory work.

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Monochloroacetic Acid in Wine

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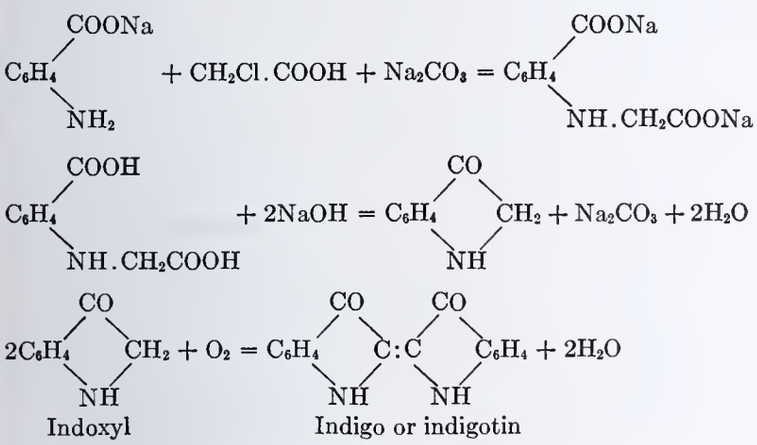
THE detection of monochloroacetic acid in the analysis of beverages brings into play one of the most renowned and historical syntheses of organic chemistry, the manufacture of synthetic indigo. Monochloroacetic acid is a basic material used in one method of its manufacture; anthranilic acid is condensed with monochloroacetic acid to produce phenylglycine-*o*-carboxylic acid, and the resultant product when heated with alkali is transformed into indoxyl, which on being made alkaline in the presence of air yields indigo. This process is followed in the detection of monochloroacetic acid in wine; the acid is extracted and purified and synthetic indigo is produced. Considering the complexity of the manipulation, the results obtained with exceedingly small quantities of the acid are excellent. At the present writing, indigo has been produced from 4.2 grams of the acid in 100 liters of wine or 42 parts per million by the use of a 200-ml. sample.

The steps in the formation of indigo from monochloroacetic acid are (*1*)



Methods for both the qualitative and quantitative determination of monochloroacetic acid in wine seem to be lacking, but Wilson (*3*) has developed a quantitative method for nonalcoholic beverages, and Chernoff (*2*), one for its qualitative detection in salad dressing. These were used as a basis for the method for its detection in wine.

In order to determine whether or not Wilson's method is applicable to wine, more than 100 authentic samples of wine were analyzed and in none of them was a trace of chlorine or chlorine compound extracted. Known quantities of monochloroacetic acid were then introduced into some of the samples and the chlorine compounds which were extracted corresponded to 94 per cent or more of the acid added.

The qualitative analysis of wine containing monochloroacetic acid is much more difficult than the quantitative determination. In extracting the very small quantities of the acid which may be present, there are also extracted wine congenerics which contaminate the acid to such an extent that Chernoff's method cannot be successfully followed without removing the interfering substances.

In the procedure outlined, the conditions of evaporation and heating of the residue at a high temperature for a few seconds which are prescribed permit attainment of consistent results when small quantities of the acid are present.

Detection of Monochloroacetic Acid in Wine

REAGENTS. Anthranilic acid reagent. Dissolve 1 gram of anthranilic acid in 50 ml. of water containing 0.3 gram of sodium hydroxide (7.5 ml. of *N* sodium hydroxide + 42.5 ml. of water).

Caustic soda solution. Dissolve 10 grams of caustic soda in 10 ml. of water.

Anhydrous sodium carbonate, C. P.; ether, C. P.; and benzene, thiophene-free, which meets A. C. S. specifications.

A dropper, 20 cm. (8 inches) long, with orifice 2 mm. in diameter. Rubber bulb, 2-ml. capacity.

METHOD. Place 200 ml. of sample, to which have been added 2 ml. of concentrated sulfuric acid in a continuous extractor and extract rapidly with ether for 2 hours, using a 500-ml. round-bottomed flask for the receiver, and preferably heating on a water bath over an electric heater. Pour the ether from the extraction chamber through a dry filter paper into the round-bottomed flask by tilting the extractor to drain as much of the ether as possible into the flask without contamination by the wine solution. Discard the wine and clean the extraction chamber. Add 5 ml. of distilled water to the ether in the flask, shake vigorously, and distill the ether back into the extraction chamber, which is cooled by being placed in a beaker of ice water. This usually leaves 30 to 35 ml. of alcoholic solution in the flask.

Place the alcoholic solution in a 15-cm. (6-inch) porcelain evaporating dish and evaporate it in the cold by use of an electric fan until approximately 10 ml. of liquid remain. Place the liquid in a separatory funnel, add 25 ml. of ether, shake vigorously for 5 minutes, allow to settle for 5 minutes, draw off the aqueous solution, and decant the ether into a 150-ml. beaker. Evaporate the ether in the cold by use of an electric fan, to the residue add 25 ml. of benzene, stir, and pour into a 50-ml. glass-stoppered Erlenmeyer flask, washing out traces of the residue by pouring back the benzene several times from the flask. Add 3 drops of water to remove the last remaining portions of residue and add them to the flask. Shake vigorously for 5 minutes, allow to settle for 5 minutes, decant the benzene into a 150-ml. beaker, evaporate in the cold by the use of an electric fan, and to the benzene-free residue add 2 ml. of the anthranilic acid reagent and 0.03 gram by actual weight of anhydrous sodium carbonate, and stir. This solution should be alkaline; if it is not, add another 0.03 gram of the sodium carbonate. No more than 0.06 gram of sodium carbonate should be added and the second portion should not be added unless absolutely necessary.

Pour the liquid into a 15 × 1.56 cm. (6 × 0.625 inch) test tube having a fully rounded bottom and heat in a boiling water bath for 30 minutes. Place in a drying oven at 120° to 130° C., with the test tube reclining at a 45° angle, and evaporate nearly to dryness or until only 1 drop of liquid remains. With the long dropper, add 2 drops of the caustic solution and replace in the oven to dry, keeping the contents in one spot of the test tube, which is inclined at a 45° angle. Dry at 120° to 130° C. for approximately 60 minutes, remove from the oven, and without cooling, plunge the test tube into a molten solder bath maintained at 305° to 312° C. for approximately 30 seconds. The temperature of the solder bath and the time of heating for this reaction should be strictly observed to prevent decomposition of the phenylglycine-*o*-carboxylic acid. As the alkali melts, the contents of the tube may assume an orange color, the depth of color depending upon the amount of reacting materials. Remove the tube from the bath, cool immediately, add at once 8 to 10 ml. of water, and shake in contact with the air. A green to deep blue color will become apparent almost immediately if indigo has developed. After approximately 10 minutes, acidify the liquid with *N* hydrochloric acid, shake thoroughly to subdivide the precipitate, and pour through a folded filter paper, washing with approximately 100 ml. of water to remove all the acid. Dry at room temperature the filter paper which contains the indigo.

In the first extraction of the sample with ether, in addition to any monochloroacetic acid present, many of the wine congenerics are partly or completely extracted, such as glycerol, tartaric acid, alcohol, higher alcohols, esters, and coloring material. The second extraction with ether removes 80 per cent of any monochloroacetic acid present and some of the wine congenerics, leaving the bulk of them behind in the small amount of aqueous solution. To purify the acid further, benzene is employed. Chloroform was tried as a solvent, but it removed too many impurities to give

Table I. Determination of Monochloroacetic Acid

Wine	Date of Analysis	Size of Sample	Alcohol	Monochloroacetic Acid
		Ml.	%	*P. p. m.*
Sauterne	June 13, 1942	200	13	90.0
	July 27, 1942	200		90.0
	January 4, 1943	200		90.0
Dry white	June 5, 1942	200	13	230.0
	January 5, 1943	100		226.8
Champagne dosage solution	August 13, 1942	200	14	406.0
	August 20, 1942	25		411.0
	January 5, 1943	50		396.8

satisfactory results. Benzene removed small amounts of the acid in a fairly pure state, the main deterrent to extraction being the presence of water. The importance of having as little water as possible present at this point in the procedure is shown by the fact that when a sample containing 20 mg. and one containing 40 mg. of the acid are extracted with benzene, 26 per cent of it is removed in each case, whereas if 7 ml. of water are present only 5 per cent of the acid is extracted with benzene. When as little as 2 mg. of the acid is separated from the wine in a fairly pure state for the final reactions, positive results are obtained with the test.

To obtain consistent results in the detection of minute quantities of monochloroacetic acid in wine, the method must be carefully followed in several details—the test tube kept at a 45° angle at all times, so that the residue is concentrated in one small spot; the initial evaporation in the test tube continued until only 1 drop of liquid remains; and the solid alkaline mass heated for 1 hour or more for complete desiccation, so that the final reaction at 305° to 312° C. may be obtained in the required time of approximately 30 seconds. Occluded moisture causes the compact mass to swell and allow too much air to take part in the reaction, and prevents the sudden reaching of the reaction temperature, resulting in prolonged heating and destruction of the phenylglycine-*o*-carboxylic acid.

In some of the wine, an emulsion formed during the first extraction with ether. Since no way was found to prevent it, the procedure was to continue the extraction for the required time, allowing the emulsion to be carried over into the receiver. The ether was distilled off as usual, and the solution containing the monochloroacetic acid was made up to the original volume with acidified water and reextracted with ether. This time no emulsion formed or not enough to be carried into the receiver.

Results so far obtained in this laboratory show that the presence of sulfur compounds in wine as normally used for preservative purposes has no effect on the determination of monochloroacetic acid and the presence of the acid does not interfere with the quantitative determination of sulfur dioxide therein.

In addition to various types of wine, the juice expressed from unripe grapes was analyzed. Samples of 175 ml. of juice from each of two varieties were analyzed by the qualitative method and in both cases the results were negative.

The chlorine compounds naturally present in normal wines are not extracted by ether; therefore any chloride in the ether extract must come from foreign material added to the wine.

Quantitative Determination

The following method is that of Wilson (*3*), with a few minor changes necessary to make it applicable to the small quantity of the acid found in wine.

Place 200 ml. of wine and 2 ml. of concentrated sulfuric acid in a continuous extractor and extract with ether for 2 hours. Disconnect the apparatus and drain through a dry filter paper into the flask as much of the ether as possible from the extraction chamber, add 25 ml. of *N* sodium hydroxide, and distill the ether back into the extraction chamber, which has been emptied and is cooled by being placed in a beaker of ice water. Disconnect the flask and digest on a steam bath for 2 hours. Cool, and add 50 ml. of water, 8 ml. of nitric acid, and 20 ml. of 0.1 *N* silver nitrate. Boil the solution gently for several minutes, allow to cool in the dark for 30 to 60 minutes to settle the precipitate, filter off the silver chloride, wash the filter paper thoroughly with water, and titrate the excess of silver nitrate with 0.1 *N* thiocyanate solution, using 5 ml. of 8 per cent ferric alum solution as indicator.

In connection with the process of removing wine extractives for the detection of monochloroacetic acid, the quantitative determination of the acid by continuous extraction with chloroform was tried on a commercial wine in which 9 mg. of the acid per 100 ml. of wine were obtained by ether extraction, using 200 ml. of sample. The chloroform extracted 7.1 mg. of the acid during the first 1.5 hours and 11.3 mg. during a further 1.5-hour extraction, making a total of 18.4 mg. from 200 ml. of sample. However, no advantage was gained by the use of chloroform instead of ether.

The range of quantities of monochloroacetic acid so far

Table II. Analyses of Wines and Champagnes

Mfg.	Wine	Alcohol	Storage	Monochloroacetic Acid	SO_2 Free	SO_2 Total	Indigo Produced	Chlorine Due to $CH_2ClCOOH$	Chlorine Due to Wine	Chlorine Due to Total
		%		*P. p. m.*	*P. p. m.*	*P. p. m.*		*Gram/100 ml.*		
A	Sweet white	13	Tank	230	...	66.0	Yes	0.0085	0.0053	0.0138
	Sweet white	13	Tank	270	...	...	Yes	0.0101	0.0059	0.0160
	Sweet red	13	Tank	230	...	87.0	Yes	0.0085	0.0053	0.0138
	Sauterne	13	Bottle	90	...	209.9	Yes	0.0034	0.0030	0.0064
B	Sauterne	13	Bottle	90	...	74.2	Yes	0.0034	0.0059	0.0093
	Claret	13	Bottle	90	...	...	Yes	0.0034	0.0076	0.0110
C	Medium dry	12	Bottle	None	...	...	No	0.0000	0.0039	0.0039
	Sweet white	13	Bottle	None	26.0	256.0	No	0.0000	0.0057	0.0057
D	Dry white	..	Tank	None	...	...	No	0.0000	0.0036	0.0036
	Dry red	..	Tank	None	...	...	No	0.0000	0.0046	0.0046
E	Chateau type	..	Tank	None	253.6	771.8	No	0.0000	....	....
	Sauterne	12	Bottle	None	126.5	289.3	No	0.0000	....	....
F	Champagne	12	Bottle	187	...	...	Yes	0.0071	....	....
	Champagne	12	Bottle	137	...	...	Yes	0.0052	....	....
	Wine dosage solution	14		406	...	...	Yes	0.0153	....	....
	Sparkling wine	13	Bottle	230	...	...	Yes	0.0085	....	....
G	Dry white (6 samples)	13	Tanks	42 on each	...	...	Yes	0.0016	....	....

extracted from commercial wines and champagnes in this laboratory extends from 42 to 400 parts per million, which have fallen into four average groups: 42, 90, 150, and 250 parts per million.

To determine whether or not monochloroacetic acid is decomposed in wine on long standing, three samples were analyzed at various intervals of time. The results given in Table I not only show that the acid is stable in wine, but also check the accuracy of the method, since consistent figures were obtained with varying amounts of sample.

Table II contains some of the results of the analysis of various wines and champagnes made during the course of the work.

The total chlorine was determined by the official A. O. A. C. method for chlorine in wines, by the addition of sodium carbonate and evaporation to dryness. Two hours or more are required for the determination and Wilson has shown that monochloroacetic acid is completely hydrolyzed in that length of time, according to the equation $2CH_2Cl.COOH + Na_2CO_3 + H_2O = 2CH_2OH.COOH + 2NaCl + CO_2$. Therefore the figure for total chlorine in the wine includes that derived from any monochloroacetic acid present.

The chlorine due to monochloroacetic acid was determined by Wilson's method and that due to the wine was found by difference. The chlorine content due to the wine itself, as shown in Table II, is normal for the types of wine represented.

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A New Selective Reagent for Lithium

Application to the Rapid Volumetric Estimation of Lithium in the Presence of Potassium and Sodium

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LITHIUM is quantitatively precipitated as a complex periodate by a strongly alkaline potassium periodate solution. Such a solution may be made the basis of selective methods for the detection or determination of lithium, since with proper adjustment of alkalinity the other alkali metals do not form insoluble periodates. By systematic experiments with a series of trial reagents it was found that a reagent of the following composition gave the best results: potassium hydroxide, 24 grams; potassium metaperiodate, 10 grams; water, 100 ml.

The potassium hydroxide is first dissolved in the water, and after the solution has become nearly cool the potassium metaperiodate is dissolved. Simultaneous dissolution of the two solids is not a satisfactory procedure, since slight decomposition of the periodate then often occurs, apparently as a consequence of heat developed by the dissolution of the potassium hydroxide. The reagent undergoes slight decomposition by the action of light and should preferably be kept in a dark bottle. Because of its high alkalinity it should be placed in a paraffined container unless all of it is to be used within a very short time. When properly prepared and bottled, this reagent is stable for at least a month.

Qualitative Behavior

For delicate results in the detection of lithium, both the volume of test solution and the volume of reagent must be properly restricted. The test solution should be reduced to a volume of 1 ml. or less, and an equal volume of reagent should be added. With 1 ml. of each, 0.5 mg. of lithium present as chloride yields a distinct precipitate in one minute in the cold, and if the mixture is warmed to about 70° C., a distinct precipitate is produced by 0.1 mg. of lithium. The delicacy of the test is but little reduced by the use of larger volumes of reagent up to 5 ml., but is greatly reduced by the use of larger volumes of test solution.

As much as 50 mg. of sodium or ammonium, or 100 mg. of potassium, present as chloride does not yield a precipitate when the volume of test solution and reagent is each 1 ml. The presence of potassium, sodium, or both does not reduce the sensitivity of the test, but the presence of ammonium in appreciable concentration does. For example, in the presence of 50 mg. of ammonium no precipitate is produced when 1 ml. of reagent is added to 1 ml. of test solution containing 0.5 mg. of lithium. For best results, therefore, ammonium should be removed before testing for lithium with this reagent. Excess ammonium may be readily removed by boiling the test solution with a slight excess of concentrated potassium hydroxide solution, and the test may be made directly on the boiled solution, since some potassium hydroxide in the test solution does not interfere with the lithium reaction.

Because of the nature of the reagent, metals other than alkali metals must obviously be absent. Common anions such as chloride, nitrate, or sulfate do not interfere. Moreover, free strong or weak acids, when not present in high concentration, do not interfere, though when such acids are present some free iodine may be liberated on the first addition of the reagent to the test solution, but this disappears when the solution becomes basic. It is better in practice, however, to neutralize any free acid with potassium hydroxide solution before adding the reagent. Numerical data on interference are given under the discussion of the quantitative application of this reagent.

Properties and Composition of the Precipitate

The precipitate formed by this reagent is finely divided but may be separated by filtration without much difficulty, especially when the precipitation is performed slowly at an elevated temperature. It is soluble in water, in acid

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Table I. Analyses of Precipitates Formed by Addition of Periodate Reagent to Solutions of Lithium Chloride

Preparation	Average Lithium Content	Average Iodine Content	Atomic Ratio of Lithium to Iodine
	%	%	
I	11.13	46.62	4.37
II	12.27	49.10	4.57
III	11.87	47.01	4.62
IV	12.42	48.34	4.70

Key to preparations:
I. Precipitated cold and dried in air at room temperature.
II. Precipitated cold and dried at room temperature over P_2O_5.
III. Precipitated hot and dried in air at room temperature.
IV. Precipitated hot and dried in air at 90° C.

solutions, or in weakly alkaline solutions. Only in strongly alkaline solutions does it have a low solubility in aqueous media. However, when precipitated in and washed with a strongly alkaline solution, considerable alkali is firmly retained by the precipitation, probably in large part by adsorption. Though but slightly soluble in the common organic solvents miscible with water, most of these react with it to an appreciable extent. Hence, it is difficult to isolate this precipitate in a pure state for the purpose of studying its composition. By precipitating a large enough quantity and washing it sparingly with small portions of distilled water, enough to provide samples for analysis may be isolated.

Actually, in preparing the four samples for the analyses shown in Table I, 200-mg. quantities of lithium were precipitated with 40 ml. of reagent. The precipitate separated by filtration was transferred to a centrifuge, and washed with 10 successive 5-ml. portions of distilled water. Lithium was determined either gravimetrically as the sulfate or volumetrically by the method described below. Iodine was determined by titration with standard arsenite solution after adding potassium iodide to the properly prepared solutions of the samples.

There are not only differences in the percentage composition of these samples, but no integral stoichiometric relationship exists between the proportions of lithium and iodine. The differences in composition are evidently due in part to differences in the extent to which the samples were dried, but all differences in composition cannot be ascribed to differences in the method of drying. It is not unlikely that some alteration of the original composition of the precipitates occurred on washing them with water, and this may account in part for the observed differences in composition. However, neither the method of drying nor the method of washing can account for the lack of integral stoichiometric ratio between the proportions of lithium and iodine, since the same lack of integral ratio was found to exist in precipitates which had neither been washed with water nor dried. Moreover, as indicated by Table I and as established definitely in the development of the volumetric method described below, the atomic ratio of lithium to iodine is lower in precipitates formed at room temperature than in precipitates formed at higher temperatures. Evidently, the precipitate produced by this reagent is a mixture of lithium periodates and does not consist of a single compound. However, as shown by the quantitative studies described below, this mixture is so constant in composition when formed under fixed conditions that its lithium content may be accurately determined from a measurement of its iodine or periodate content. The ratio of lithium to iodine in the precipitate is much more variable after washing with water and drying than in the wet precipitate separated from solution and washed with strong alkali solution. Because of the difficulty of washing and drying this precipitate and because of its uncertain composition when washed and dried, it apparently is useless for the gravimetric determination of lithium. It may, however, be made the basis of a rapid and satisfactory empirical method for the volumetric estimation of this element.

Quantitative Application

By properly restricting the volume of the solution in which lithium is to be determined and the volume of reagent used, quantities of lithium as small as 0.1 mg. may be quantitatively precipitated, and for convenience in manipulation 50 mg. is about the maximum amount that should be precipitated, though somewhat greater amounts may be precipitated and successfully determined. After precipitating, filtering, and washing in the manner detailed in the procedure, the precipitate is dissolved in dilute sulfuric acid and the liberated periodate is determined by titration, which may be done in several different ways. For example, potassium iodide may be added to the acid solution, and the liberated iodine may be determined by titration with a standardized 0.1 *N* thiosulfate solution. Because of the very large ratio between volume of thiosulfate solution and weight of lithium in the precipitate, this method of titration is unusually suitable for the determination of amounts of lithium not exceeding 10 mg., but is not suitable for the determination of larger amounts of lithium. For such determinations it is better to buffer the acid solution of the precipitate with an excess of borax or sodium bicarbonate, add potassium iodide, and then titrate with a standardized 0.1 *N* sodium arsenite solution. The solution of the precipitate may also be titrated with arsenite by adding the potassium iodide first to the acid solution and afterward adding the buffer. In this way the ratio of volume of titrating solution to weight of lithium is as favorable as in a thiosulfate titration. The validity of this variation in the usual method of titrating periodate with arsenite solution was established by appropriate experiments. Thus a single standardized arsenite solution may be used for the titration of both large and small amounts of lithium in the range in which this method is applicable.

Table II. Trial Determinations with Lithium Alone

Lithium Present	Volume of 0.1040 *N* Arsenite Solution Required	Lithium Found	Error
Mg.	*Ml.*	*Mg.*	*Mg.*
Cold Precipitation and Titration with Standard Arsenite Solution			
0.5	0.25	0.4	−0.1
1.0	0.55	0.9	−0.1
5.0	3.00	5.0	±0.0
10.0	6.02	9.9	−0.1
20.0	12.32	20.2	+0.2
25.0	15.26	25.0	±0.0
50.0	30.70	50.3	+0.3
50.0	30.40	49.7	−0.3
Hot Precipitation and Titration with Standard Arsenite Solution			
0.5	0.27	0.4	−0.1
1.0	0.55	0.9	−0.1
2.0	1.17	2.0	±0.0
5.0	2.93	5.0	±0.0
10.0	5.93	10.1	+0.1
20.0	11.87	20.2	+0.2
25.0	14.84	25.3	+0.3
50.0	29.34	50.0	±0.0

Table III. Trial Determinations with Lithium Alone

(Hot precipitation and titration with standard thiosulfate solution)

Lithium Present	Volume of 0.1034 *N* Thiosulfate Solution Required	Lithium Found	Error
Mg.	*Ml.*	*Mg.*	*Mg.*
0.1	0.12	0.05	−0.05
0.2	0.43	0.2	±0.0
0.5	1.12	0.5	±0.0
1.0	2.34	1.0	±0.0
2.0	4.70	2.0	±0.0
5.0	11.85	5.0	±0.0

Table IV. Trial Determinations of Lithium in the Presence of Sodium

Volume of Reagent	Volume of Test Solution	Sodium Present	Lithium Present	Lithium Found	Error
Ml.	*Ml.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*
		Cold Precipitation			
1	1	50	0.5	0.3	−0.2
2	2	150	0.5	0.3	−0.2
1	1	50	5.0	5.2	+0.2
1	1	50	5.0	5.1	+0.1
5	1	50	5.0	5.0	±0.0
2	2	100	5.0	5.0	±0.0
3	3	150	5.0	5.0	±0.0
10	2	200	5.0	6.1	+1.1
5	1	50	50.0	49.7	−0.3
10	2	150	50.0	50.5	+0.5
		Hot Precipitation			
1	1	10	0.5	0.5	±0.0
2	2	30	0.5	0.5	±0.0
1	1	50	0.5	1.0	+0.5
1	1	10	5.0	4.9	−0.1
1	1	20	5.0	8.0	+3.0
2	2	20	5.0	4.9	−0.1
1	1	30	5.0	9.9	+4.9
2	2	30	5.0	4.9	−0.1
1	1	40	5.0	10.4	+5.4
5	1	50	5.0	4.9	−0.1
5	1	50	50.0	49.5	−0.5
2	2	100	5.0	25.0	+20.0
3	3	150	5.0	35.0	+30.0

Procedure

From the chloride, nitrate, perchlorate, or sulfate solution containing not more than 50 mg. of lithium remove all metals except those of the alkali group. Remove ammonium also, if more than a few milligrams are present. Reduce the volume of the solution to 2 ml., finally placing it in a beaker of not more than 50-ml. capacity. Immerse the bottom half of the beaker in a water bath maintained at 60° to 70° C., and after a few minutes add the special periodate reagent dropwise with constant swirling at a rate not exceeding one drop every 5 seconds until 2 ml. have been added. If a heavy precipitate forms add 3 ml. more of reagent in the same way. If the precipitate is very heavy, as shown by the formation of a mixture that does not flow freely, add another 3 to 5 ml. of reagent. Allow the precipitated solution to digest at 60° to 70° C. for 20 minutes and then filter through a Gooch crucible fitted with a moderately thick asbestos pad. Wash the precipitate with 4 successive 2-ml. portions of 3 to 5 *N* potassium hydroxide added slowly from a pipet. If more than 20 mg. of sodium is present, precipitate, filter, and wash in the same way but at room temperature. Transfer the pad and precipitate to a 250-ml. beaker with the aid of distilled water, and add 5-ml. of *N* sulfuric acid in order to ensure complete solution of the precipitate.

Titrate the periodate in the solution by means of a standardized sodium thiosulfate or arsenite solution. If thiosulfate is used adjust the acidity, add potassium iodide, and titrate in the usual way. If arsenite is used and the amount of lithium is relatively high, add an excess of borax or sodium bicarbonate as a buffer before adding the potassium iodide and titrating. If the amount of lithium is low, add the potassium iodide before the buffer and then titrate. Standardize the titrating solution on a known amount of lithium by exactly the same procedure as used in the determination.

In Tables II and III is shown the degree of accuracy obtainable when this procedure is applied to pure lithium chloride solutions. The results of Table II are based upon the results for the 50-mg. quantity taken as a standard. For those of Table III, the 5-mg. quantity was taken as the basis. Although the accuracy is not high, it is satisfactory from the standpoint of practical analysis. The results in these tables illustrate well the different volumes of the same titrating solution required for a given quantity of lithium when the precipitation is made at different temperatures, and also the different volumes of arsenite and thiosulfate solution required for a given quantity of lithium.

The results in Table IV indicate the effect of sodium on the accuracy of the determination. These results were also obtained on chloride solutions. It is evident that when precipitation is done at room temperature interference occurs only with the largest amounts of sodium that may possibly be present, whereas at an elevated temperature interference occurs with much smaller amounts of sodium. The reason for this difference apparently is that a periodate containing sodium precipitates out at the elevated temperature. When more than a decigram or two of sodium is present it is advisable to precipitate at room temperature. Precipitation at an elevated temperature produces a precipitate that may be filtered and washed more easily and rapidly, but, to avoid gross errors, precipitation should always be made at room temperature whenever doubt exists as to the amount of sodium present.

As shown by Table V, considerable amounts of ammonium lead to low results, apparently because ammonium in high concentration increases the solubility of the lithium precipitate. However, relatively small amounts do not cause an appreciable error. Table VI indicates that lithium may be successfully determined by this method in the presence of various substances, some of which will commonly be present in the solution in which the lithium is to be determined when this solution is a filtrate from other determinations or separations.

In spite of the empirical nature of the method lithium may be successfully determined by its use in the presence of sodium and certain other substances which ordinarily interfere with the quantitative determination of this element. As far as the authors are aware, this is the only method by which lithium may be determined in the presence of sodium with rapidity and satisfactory accuracy.

Table V. Trial Determinations of Lithium in the Presence of Ammonium

Volume of Reagent	Volume of Test Solution	Ammonium Present	Lithium Present	Lithium Found	Error
Ml.	*Ml.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*
1	1	50	5.0	4.8	−0.2[a]
3	1	50	5.0	5.1	+0.1[a]
2	2	100	5.0	4.8	−0.2[a]
3	3	150	5.0	3.7	−1.3[a]
2	2	50	5.0	5.1	+0.1[b]
3	1	50	5.0	4.9	−0.1[b]
5	1	50	5.0	5.0	±0.0[b]
3	1	100	5.0	4.1	−0.9[b]

[a] Hot precipitation.
[b] Cold precipitation.

Table VI. Trial Determinations of Lithium in the Presence of Various Substances

Substance Added to Test Solution	Volume of Reagent	Volume of Test Solution	Lithium Present	Lithium Found	Error
	Ml.	*Ml.*	*Mg.*	*Mg.*	*Mg.*
0.05 ml. of concentrated HCl	3	1	10.0	10.1	+0.1
	3	1	10.0	9.9	−0.1
0.1 ml. of 60% $HClO_4$	3	1	10.0	10.0	±0.0
	3	1	10.0	10.0	±0.0
1.0 gram of KNO_3	9	3	10.0	9.9	−0.1
	9	3	10.0	10.0	±0.0
0.3 gram of K_2SO_4	9	3	10.0	10.0	±0.0
	9	3	10.0	10.0	±0.0

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The Rolling Ball Viscometer

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A study of the system of the inclined tube and rolling ball as applied to the measurement of viscosity is described. Dimensional analysis was used to derive general relations between the variables involved and the simple calibration for the rolling ball viscometer in the streamline region of fluid flow. The coefficient of the calibration equation may be calculated from the dimensions of the instrument with the aid of an experimentally determined empirical factor. By using the equations given, the useful range of the rolling ball viscometer may be predicted without experimental calibration or an instrument may be designed for measurements over any desired range of viscosity. An empirical correlation is given which allows viscosity to be estimated from data taken on the viscometer in the turbulent region of fluid flow. The effect of temperature changes on the viscometer and its calibration is discussed.

FOR many years the system of the inclined tube and rolling ball has been used as an empirical instrument for viscosity measurement without complete knowledge of the general relations existing between the variables involved. The instrument has been used because it is more easily adaptable for measurements in enclosed systems. It has many advantages, which may be listed as follows:

1. The apparatus can be extremely simple.
2. Only a small sample of material is required.
3. Visual observation in glass apparatus is possible even with opaque liquids, since the ball is in contact with the tube at one point.
4. The system possesses great flexibility, with the opportunity of changing one or more of the variables: tube diameter, ball diameter, angle of inclination, ball density, and rolling distance of ball.

The use of the system of the inclined tube and rolling ball as a viscometer was first suggested by Flowers (*7*) and was studied by Hersey (*8*), who evolved by dimensional treatment the manner of correlation of the variables involved. A calibration first used by Hersey and Shore (*9*) consisted of a plot of the equation

$$\beta \frac{\mu}{\rho\sqrt{\frac{\rho_s}{\rho} - 1}} = z\sqrt{\frac{\rho_s}{\rho} - 1} \qquad (1)$$

Except at high rolling velocities this relation was linear, and the line extended passed through the origin.

Sage (*13*) described the use of the system in measuring the viscosity of hydrocarbon solutions. He used a calibration of the form

$$\mu = b\, z\, (\rho_s - \rho) \qquad (2)$$

which may readily be derived from Equation 1. This relation also departed from the linear function through the origin, but only for viscous fluids and low rolling velocities.

Sage and Lacey (*14*) measured the viscosity of hydrocarbon gases in a similar apparatus and worked in the turbulent region of flow to a large extent. The value of constant b in Equation 2, which represented the calibration in the streamline region only, was obtained from observations on known liquids. Using other fluids for the turbulent region, but still calculating viscosity by Equation 2, a viscosity-ratio correction factor obtained for each fluid was plotted against a function proportional to Reynolds number. By use of this plot applicable to the one instrument only, the viscosity of fluids flowing with turbulence was calculated by the method of successive trials.

Block (*5*) has recently suggested the addition of a term containing an empirically derived exponent to the calibration Equation 1 to effect agreement of the equation with experimental results in the turbulent region.

Hoeppler (*10*) reported the results of experimental work on the eccentric fall of large spheres in a tube inclined at an angle of 80°. In suggesting that this arrangement of the inclined tube and rolling ball be used as a viscometer, he too employed Equation 2 as a calibration. The commercial instrument bearing his name uses a short, nearly vertical glass tube of large diameter (16 mm.) and close fitting balls of either glass or steel.

No general study of the system of the inclined tube and rolling ball has been reported. An experimental investigation of the system was therefore made on tubes from 6 to 10 mm. in diameter with balls of aluminum, steel, and brass ranging in size from 85 per cent to the full tube diameter. The general correlation obtained verified the viscometer calibration, Equation 2, and in addition indicated a method by which the unknown coefficient, b, could be calculated from the dimensions of the apparatus with the aid of an empirical correlation.

Nomenclature

b, c = proportionality constants
C = coefficient, defined by Equation 15
d = diameter of ball, cm.
D = diameter of tube, cm.
f = resistance factor $= R/(h^2\, \rho\, u^2)$, dimensionless
f_c = resistance factor at critical velocity
F = force, gram cm. per second^2
g = acceleration of gravity = 980 cm. per second^2
h = equivalent diameter of annular space between ball and tube, defined by Equation 7, cm.
K = correlation factor, dimensionless
L = length, a fundamental dimension, cm.
M = mass, a fundamental dimension, grams
R = driving force on ball or resistance of fluid to motion of ball, defined by Equation 8, gram. cm. per second^2
Re = Reynolds number $= (h\, u\, \rho)/\mu$, dimensionless
Re_c = Reynolds number at critical velocity
t = temperature, ° C.
T = time, a fundamental dimension, seconds
u = average fluid velocity through annular space between ball and tube, cm. per second
V = terminal rolling velocity of ball, cm. per second
z = time of roll, seconds
α_b = linear coefficient of thermal expansion of ball material, per ° C.
α_t = coefficient of expansion of tube, per ° C.
β = coefficient in Equation 1
δ = prefix indicating derivative
Δ = increment of change of variable
θ = angle of inclination of tube to the horizontal
μ = viscosity of fluid, grams per cm. per second
μ_0 = viscosity of fluid calculated when Equation 14 is used in the turbulent region, gram per cm. per second
π = 3.1416
ρ = density of fluid, grams per cc.
ρ_s = density of ball, grams per cc.
ϕ = symbol for "is function of"
o = subscript denoting value at temperature of calibration of viscometer

Dimensional Analysis

There are seven variables to be considered in the analysis of the rolling ball viscometer. In addition to the fundamental units—length, L; mass, M; and time, T—force, $F = MLT^{-2}$, is considered a unit of its own kind. This can

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be done because the system is in equilibrium and in unaccelerated motion, and no use is made of the fact that where there happens to be accelerated motion, force is equal to mass times acceleration. The seven variables with symbols and dimensions are:

Variable	Symbol	Dimension
Diameter of tube	D	L
Diameter of ball	d	L
Velocity of motion	V	LT^{-1}
Density of ball	ρ_s	ML^{-3}
Density of fluid	ρ	ML^{-3}
Viscosity of fluid	μ	$FL^{-2}T$
Acceleration of gravity	g	FM^{-1}

The dimensional formula of viscosity is obtained directly from its definition of force per unit area per unit velocity gradient. The intensity of gravity is taken with the dimensions FM^{-1} because the equations of motion in this case will not use the accelerating aspect of gravitational motion but only the intensity of the force exerted by gravity upon unit mass. Because of the inclination of the tube at the angle θ to the horizontal, the effective acceleration of gravity is g sine θ.

There are seven variables and four kinds of units; therefore three dimensionless products or groups of variables must be found. Two of these groups, the ratios d/D and ρ_s/ρ, may be written immediately by inspection. The third dimensionless product, obtained by the method of Bridgman (*6*), includes five variables in the form $V^{-1}\,\mu^{-1}\,d^2\,\rho\,g$ sine θ. The final general relation is

$$\phi\,(V^{-1}\,\mu^{-1}\,d^2\,\rho\,g \text{ sine } \theta)\,\phi'\left(\frac{\rho_s}{\rho}\right)\phi''\left(\frac{d}{D}\right) = 0 \qquad (3)$$

The velocity of the ball rolling down an inclined tube is given by the equation

$$V = c\,\frac{d^2\,\rho\,g \text{ sine } \theta}{\mu}\,\phi'\left(\frac{\rho_s}{\rho}\right)\phi''\left(\frac{d}{D}\right) \qquad (4)$$

The viscosity of the fluid is expressed as a function of all the variables by the relation

$$\mu = c\,\frac{d^2\,\rho\,g \text{ sine } \theta}{V}\,\phi'\left(\frac{\rho_s}{\rho}\right)\phi''\left(\frac{d}{D}\right) \qquad (5)$$

The solution of the entire problem is obtained if the unknown functions are evaluated.

The resistance to the motion of the ball is developed by the fluid in being accelerated and decelerated in passing through the constriction between the ball and tube. The average fluid velocity through this space is related to the ball velocity by the equation

$$\frac{u}{V} = \frac{d^2}{D^2 - d^2} \qquad (6)$$

The linear dimension commonly employed in hydraulics for noncircular channels is the equivalent diameter, equal to four times the hydraulic radius, which is defined as the cross-sectional area of the channel divided by the wetted perimeter. In this system the equivalent diameter of the crescent-shaped space is theoretically

$$h = 4\,\frac{\pi}{4}\,\frac{D^2 - d^2}{\pi\,(D + d)} = D - d \qquad (7)$$

The dimensional analysis can be simplified by combining the factors d, ρ_s, ρ, and θ into a force term, the driving force on the ball, equal to the resisting force of the fluid since the ball rolls with unaccelerated motion. This term is represented by the equation

$$R = \frac{5}{7}\,g \text{ sine } \theta\,\frac{\pi d^3}{6}\,(\rho_s - \rho) \qquad (8)$$

The coefficient 5/7 is that fraction of the effective force of gravity that causes translational motion of the rolling sphere.

In the dimensional treatment of a similar problem, Awberry and Griffiths (*1*) included Reynolds number as one of the dimensionless products. In a second application of dimensional analysis to the present system, the variables used are:

Variable	Symbol	Dimension
Driving force	R	MLT^{-2}
Equivalent diameter	h	L
Density of fluid	ρ	ML^{-3}
Viscosity of fluid	μ	$ML^{-1}T^{-1}$
Velocity of fluid	u	LT^{-1}

There are now five variables and three kinds of fundamental units; therefore two dimensionless products must be found. If the Reynolds number, $(h\,u\,\rho)/\mu$, is assumed to be one dimensionless product, the other is shown to be $R/(h^2\,\rho\,u^2)$, which group will now be called the resistance factor. A general relation having only one unknown function and whose terms are capable of evaluation by experiment is written

$$\frac{R}{h^2\,\rho\,u^2} = \phi\,\frac{h\,u\,\rho}{\mu} \qquad (9)$$

Apparatus and Experimental Methods

In order to evaluate the functions of Equations 5 and 9, experimental equipment designed to permit variation of all factors was constructed.

The apparatus consisted of a precision-bore glass tube (procured from the Fish-Schurman Corp., New York, N. Y., and also available from the Fischer and Porter Co., Hatboro, Penna.) in an isothermal water bath and an automatic photoelectric device for recording the time required for a rolling ball to traverse a known distance in the tube. A photograph of a tube in its bath is shown in Figure 1.

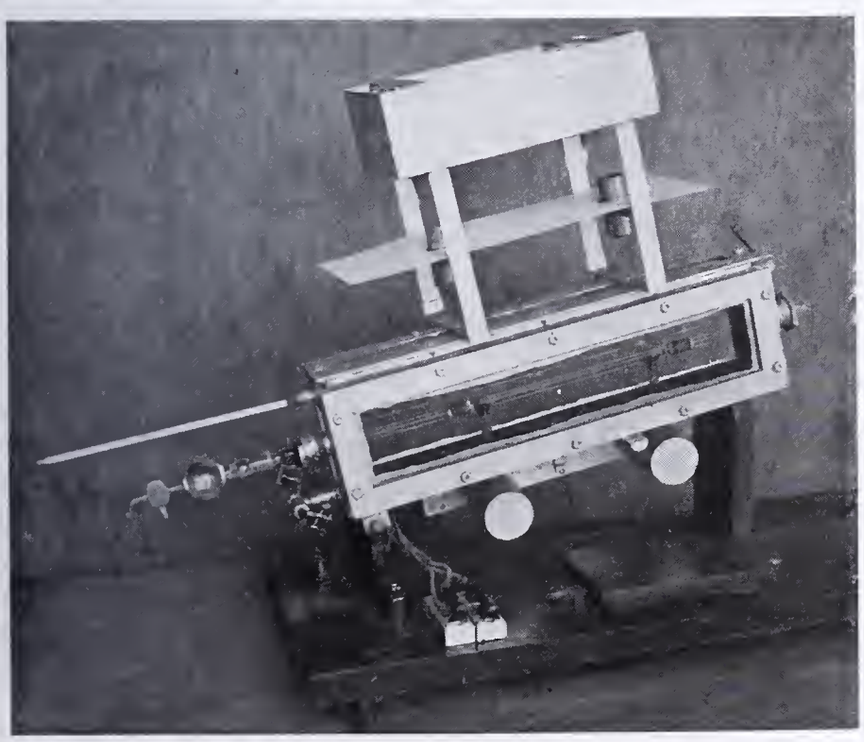

Figure 1. Experimental Apparatus, Rolling Ball in Inclined Tube

Table I. Summary of Experimental Conditions and Results

Tube	Tube Diameter	Ball Diameter	Diameter Ratio	No. of Experimental Points	Range of Reynolds No.	Critical Reynolds No.	Correlation Factor, K Calculated	Correlation Factor, K Graphical
	Cm.	*Cm.*						
I	0.5994	0.5951	0.9928	38	0.31–18.0	..	6.22×10^{-7}	.....
		0.5937	0.9905	45	0.43–32.6	..	1.16×10^{-6}	.....
		0.5861	0.9778	37	1.8–135	21.5	7.13×10^{-6}	.....
		0.5785	0.9650	38	5.1–236	18.0	.....	2.00×10^{-5}
		0.5709	0.9523	21	35–330	(15.5)	.....	4.00×10^{-5}
		0.5556	0.9269	107	8.2–511	13.0	.....	1.06×10^{-4}
		0.5144	0.8581	37	32–505	(9.8)	.....	4.17×10^{-4}
II	0.6485	0.5951	0.9177	33	20–270	(12.0)	.....	1.54×10^{-4}
		0.5785	0.8921	33	30–344	(11.0)	.....	2.78×10^{-4}
		0.5556	0.8568	99	15–448	(10.0)	.....	4.60×10^{-4}
III	0.8014	0.7950	0.9921	23	0.97–13.1	..	6.84×10^{-7}	.....
		0.7938	0.9905	61	1.03–65	..	1.02×10^{-6}	.....
		0.7525	0.9390	62	0.036–705	13.0	7.36×10^{-5}	.....
		0.7144	0.8914	237	0.0046–806	10.6	2.62×10^{-4}	.....
IV	0.9997	0.9906	0.9909	51	1.5–122	35.0	1.13×10^{-6}	.....
		0.9830	0.9832	20	18–263	24.0	.....	3.94×10^{-6}
		0.9754	0.9756	40	12–406	20.0	.....	7.15×10^{-9}
		0.9677	0.9680	21	63–537	(18.5)	.....	1.59×10^{-5}
		0.9525	0.9527	109	13–916	17.5	.....	4.65×10^{-5}
		0.9112	0.9115	31	60–936	(11.5)	.....	1.67×10^{-4}
		0.8731	0.8734	44	38–383	(10.0)	.....	3.45×10^{-4}

Values in parentheses obtained by extrapolation.

Light from two lamps was focused onto the upper surface near the ends of the glass tube. Light passing through the tube was conducted through quartz tubes to two gas-filled photoelectric cells. These cells were part of circuits which, through sensitive relays, controlled an electric chronoscope. When the light to the first photocell was interrupted by a ball rolling down the inclined tube, time measurement by the chronoscope was started. Similarly, the ball stopped the time measurement in passing through the second light beam.

The inclination of the tube and the distance traversed by the balls were measured with a cathetometer. The roll distance between the light beams was measured as the distance between the positions of stationary balls placed in each light beam at the point at which they just caused the relays to operate the chronoscope. The inclination of the tube was varied from 4° to 25° from the horizontal. The distance between the light beams was about 17 cm.

The glass tube was carefully cleaned before use. Water maintained at a constant temperature was circulated through the jacket by a pump. After the water jacket attained the desired temperature, the tube and reservoir at its lower end were filled with the liquid used. At each inclination about ten balls of each size and material were introduced into the open end and successively rolled down the tube. The balls were removed from the receiver at the lower end of the tube, the tube was refilled, and the procedure was repeated at another inclination. The roll velocity was calculated from the known distance and the average of the values of roll time.

Four Jena KPG glass tubes were used in this work. Their inside diameters were measured with plug gages. Seventeen different ball sizes were used. Steel balls are manufactured to a high degree of precision and were assumed to be their nominal diameter. Close fitting aluminum balls were specially measured by the manufacturer (Hoover Ball and Bearing Co., Ann Arbor, Mich.). Table I gives the diameter of the tubes and balls used.

Sixteen fluids were used. Except for air, water, ethyl alcohol, and solutions of ethanol and sucrose, which are accepted as standards for viscometer calibration, the viscosity of the fluids was measured in Bingham (*3*) or Ostwald capillary tube viscometers. The density of all fluids was measured experimentally. The viscosity of the fluids varied from about 0.23 to 144 centipoises, the density from 0.62 to 1.61 grams per cc. The measurements were made to an estimated precision as follows:

Tube diameter	0.08 per cent
Ball diameter	0.01 to 0.05 per cent
Ball density	0.03 per cent
Fluid density	0.02 per cent
Roll distance	0.1 per cent
Roll time	0.2 per cent
Inclination of tube	0.05 to 0.1 per cent

The major source of error was the change in viscosity of the fluid with slight changes in temperature, caused by contact of the fluid with the rolling balls. Most of the work was conducted at room temperature. At other temperatures the balls were brought to operating temperature in a separate container jacketed with the circulating water. An over-all precision in calculated rolling velocity from 0.5 to 1 per cent was obtained on measurements made under similar conditions at different times.

Experimental Results

The conditions used in the experimental work more than covered the useful range for viscosity measurement. The streamline region of fluid flow, which is the region having characteristics suitable for viscosity measurement, was covered over its most useful range. The turbulent region of flow, often used but not as advantageous for viscosity measurement, was also covered.

The turbulent region was characterized by a deviation from the relations existing in the streamline region of flow. The deviation is probably due to the inertia of the fluid, to the formation of eddy currents in the flowing fluid, or to a combination of these causes. Block (*5*) has expressed the opinion that inertia and not turbulence accounts for the deviation. No attempt was made in this work to determine the causes and only an empirical correlation was obtained.

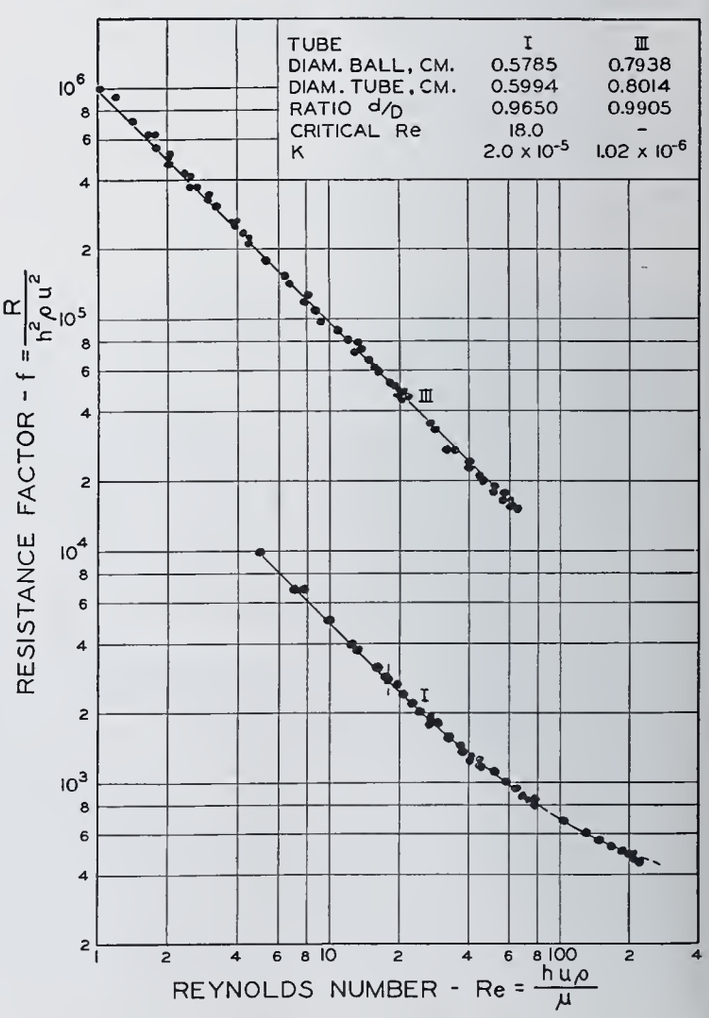

Figure 2. Typical Correlation for System Rolling Ball in Inclined Tube

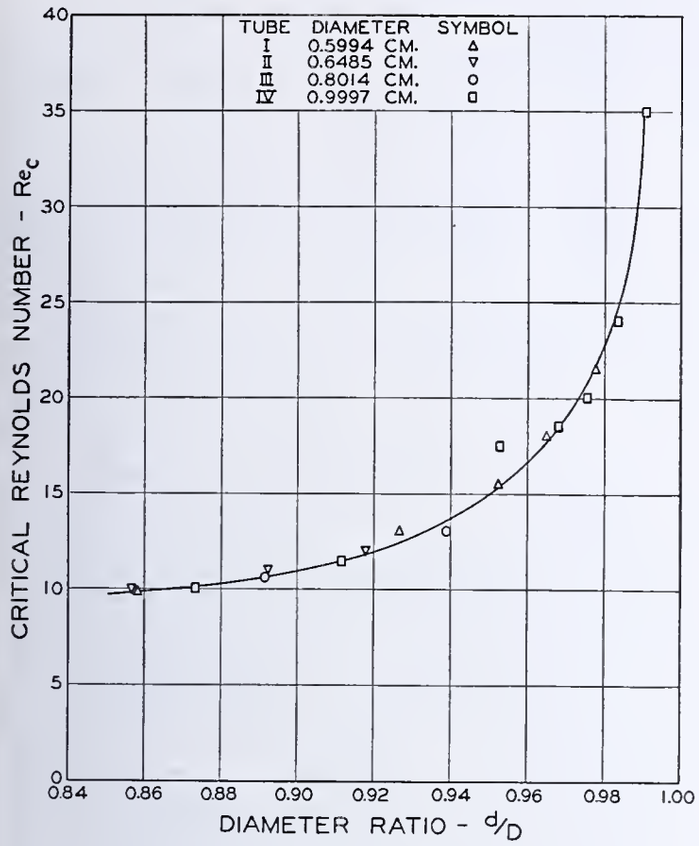

FIGURE 3. CRITICAL REYNOLDS NUMBER FOR ROLLING BALL VISCOMETER

The observed motion of the ball was fundamentally a steady rolling motion at constant velocity. Block (*4*) observed and measured the extent of sliding motion in combination with rolling in more viscous fluids at angles above 13°. Sliding was observed in this work with viscous oils at higher inclinations. With the least viscous fluids sliding was not apparent. The critical velocity at which the fluid flow changed from streamline to turbulent could not be determined by direct observation of the rolling balls. To a large extent the motion of the ball in both the streamline and turbulent regions was uniform and the data were reproducible. The limit of usefulness of the viscometer was reached before the motion of the ball became visibly irregular.

The experimental data were grouped according to ball and tube size or ratio of diameter of ball to diameter of tube, d/D. For each combination the values of Reynolds number and the resistance factor were calculated and plotted on logarithmic coordinate paper. The twenty-one plots obtained were similar in every respect to the fluid friction plot for flow through pipe. Two representative curves are shown in Figure 2.

In the region of streamline flow the experimental data fell on a straight line of slope −1 (upper curve of Figure 2). The turbulent region was represented by a smooth concave curve as shown by the lower curve. Within the limits of the experimental data all curves had the same shape. Regardless of the values of ball and tube diameter, the location of the curve on the coordinate system was dependent only on the ratio d/D.

For streamline flow the equation of the straight line through the plotted data was expressed as

$$\log \frac{R}{h^2 \rho u^2} = -\log \frac{h u \rho}{\mu} + \log \frac{1}{K} \tag{10}$$

which became Equation 11.

$$\frac{R}{h^2 \rho u^2} = \frac{1}{K} \frac{\mu}{h u \rho} \tag{11}$$

After substituting the values of the equivalent diameter, h, and the driving force, R, given by Equations 7 and 8, respectively, Equation 11 was written

$$\mu = \frac{5\pi}{42} K \frac{d^2 \rho g \sin \theta}{u} \frac{\rho_s - \rho}{\rho} \frac{d}{D - d} \tag{12}$$

By expressing the fluid velocity in terms of the ball velocity, V, the above relation became

$$\mu = \frac{5\pi}{42} K \frac{d^2 \rho g \sin \theta}{V} \frac{\rho_s - \rho}{\rho} \frac{D + d}{d} \tag{13}$$

Equation 13 is dimensionally correct and similar to Equation 5. The correlation factor, K, must be included with the term $(D + d)/d$ as a part of the function ϕ'', since it is shown to be a function of the ratio d/D.

When D, d, θ, and K are constant, Equation 13 reduces to

$$\mu = C \frac{\rho_s - \rho}{V} \tag{14}$$

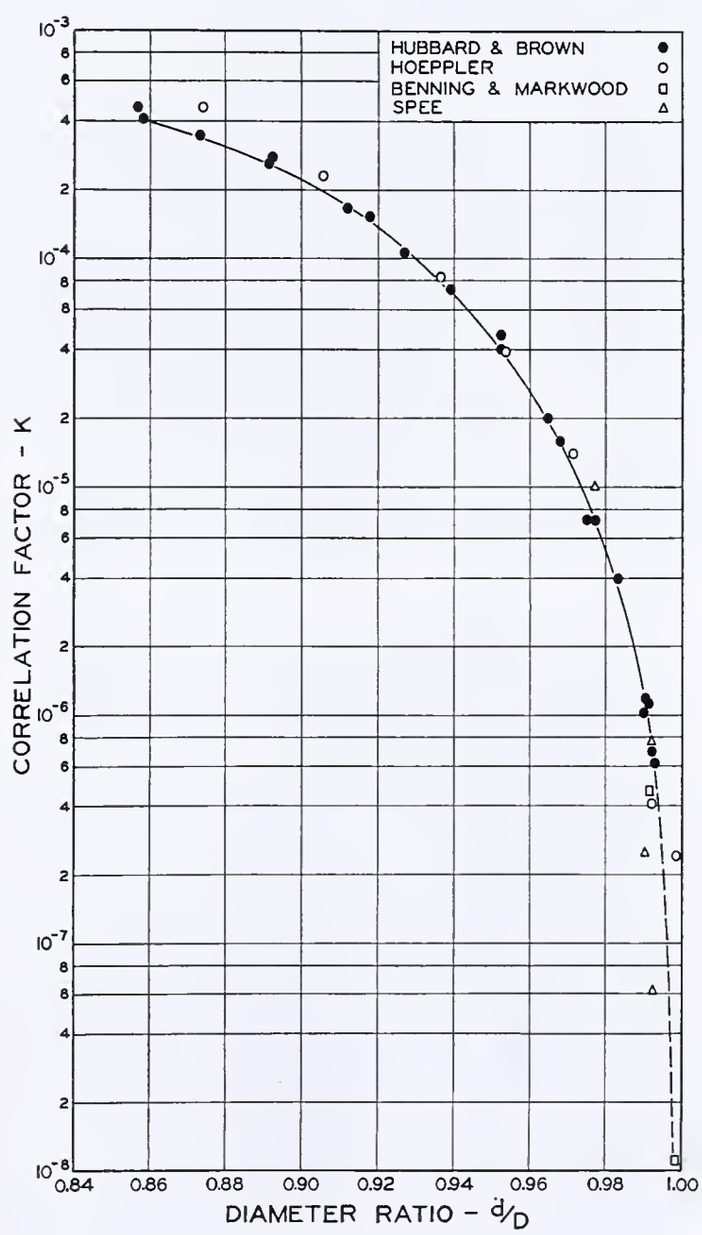

FIGURE 4. CORRELATION FOR ROLLING BALL VISCOMETER

TABLE II. CALCULATIONS FROM DATA OF HOEPPLER (*10*)

Fluid	$\left(\frac{\text{Radius of Tube}}{\text{Radius of Ball}}\right)^2$	Diameter Ratio, d/D	Ball Diameter, d	Driving Force, R	Reciprocal Velocity	Roll Velocity, V	Fluid Velocity, u	Resistance Factor, f	Reynolds Number, Re	Correlation Factor, K
		Cm.	*Cm.*	*Gm. cm./sec.*^{2}	*Sec./cm.*	*Cm./sec.*	*Cm./sec.*			
Air[a]	1.002	0.9990	1.5984	11.46×10^3	.....	0.2	100.2	3.84×10^8	1.07	2.4×10^{-7}
Castor oil[b]	1.016	0.9921	1.5874	9.844×10^3	1810	0.000552	0.0345	5.39×10^{10}	4.54×10^{-5}	4.1×10^{-7}
	1.06	0.9713	1.5541	9.235	54.7	0.01828	0.305	4.90×10^7	1.46×10^{-3}	1.4×10^{-5}
	1.10	0.9535	1.5256	8.736	20.01	0.0500	0.500	6.60×10^6	3.87×10^{-3}	3.9×10^{-5}
	1.14	0.9366	1.4986	8.280	9.92	0.1008	0.720	1.62×10^6	7.50×10^{-3}	8.3×10^{-5}
	1.22	0.9054	1.4486	7.479	3.77	0.2653	1.205	2.34×10^5	1.90×10^{-2}	2.3×10^{-4}
	1.31	0.8737	1.3979	6.722	1.96	0.5102	1.644	6.34×10^4	3.46×10^{-2}	4.6×10^{-4}

[a] Tube: diameter = 1.600 cm. assumed; inclination = 80°; sine θ = 0.9848. Ball: steel; ρ_s = 7.77. Fluid: air at 20.0° C.; $\mu = 1.81 \times 10^{-4}$; ρ = 0.0012; $\rho_s - \rho$ = 7.769.

[b] Tube: diameter = 1.600 cm. assumed; inclination = 80°; sine θ = 0.9848. Ball: steel; ρ_s = 7.775. Fluid: castor oil at 20.0° C.; μ = 9.204; ρ = 0.960 (assumed); $\rho_s - \rho$ = 6.815.

which is equivalent to Equation 2, most generally used to calculate viscosity from experimentally determined values of roll time or velocity.

Values of K were calculated from the experimental data by the method of least squares for 8 out of the 21 cases. For the remaining cases K was determined by inspection of the data. Since all the curves had the same shape, they could be superimposed by transposition. An aid to this process was a template made in the form of an average curve in the turbulent region and placed in proper relation to the straight line in the streamline region with a definite critical Reynolds number indicated at the intersection of the line and the curve. This template was superimposed on each of the plots and fitted to the experimental data in the most favorable manner. Values of the critical Reynolds number, Re_c, and the corresponding critical resistance factor, f_c, were read from the plot. The curve drawn through the data of the lower curve in Figure 2 shows the position of the template on this plot.

Values of the correlation factor, K, and the critical Reynolds number, Re_c, are given in Table I for each ball and tube combination. Most weight must be given to the calculated values of K, but many of the graphical values are based on enough points in the streamline region to assume almost equal weight. Figure 3 shows the critical Reynolds number and Figure 4 the correlation factor plotted as functions of the diameter ratio.

The value of constant C of Equation 14 can be calculated from the dimensions of the apparatus and the corresponding value of K from Figure 4 by the relation

$$C = \frac{5\pi}{42} K\, g \sin\theta\, d(D + d) \qquad (15)$$

For experimental determination of viscosity, however, it is recommended that the value of C be determined by experiment with fluids of known viscosity and density.

Equation 14 is the valid calibration for the rolling ball viscometer only in the streamline region of fluid flow. This region is defined as having a Reynolds number, $(h\ u\ \rho)/\mu$, smaller than the critical value given in Figure 3. In practice the condition of flow is checked by showing that the resistance factor is greater than the corresponding critical resistance factor. The opposite limit of the streamline region was estimated to have a resistance factor of about ten million.

For any resistance factor smaller than the value corresponding to the critical Reynolds number, there are values of Reynolds number $(h\ u\ \rho)/\mu$ and $(h\ u\ \rho)/\mu_0$, which respectively represent the abscissas of the curve and the straight line of Equation 10 extended beyond the critical Reynolds number. A ratio of these values is equal to μ/μ_0, which is the ratio of the true viscosity to that calculated from the calibration of the instrument in the streamline region. For each experimental point known to lie in the turbulent region, this ratio and the ratio of the resistance factor to the critical resistance factor were calculated. The plotted points with a curve drawn through them are shown in Figure 5. The scattering of the points is not unusual in this transition zone between the regions of streamline and turbulent flow.

The curve of Figure 5 is drawn through points obtained for relatively close fitting balls and is a general correlation from which viscosity may be calculated from data taken with the rolling ball viscometer in the turbulent region of flow. The diameter ratio will be known. The viscometer will have been calibrated in the streamline region with known fluids, or the value of the correlation factor may be read from Figure 4. The value of the critical Reynolds number may be read from Figure 3. The corresponding critical resistance factor is then calculated by substituting these values in Equation 11. Corresponding values of the resistance factor, f, and the Reynolds number, Re, may be calculated from the experimental roll velocity and the known driving force by means of Equation 11. If the resistance factor, f, is smaller than the critical resistance factor, f_c, the data were taken in the turbulent region of flow. Nevertheless the viscosity, μ_0, is calculated from Equation 14. Corresponding to the ratio of the resistance factors f/f_c, the ratio of the true viscosity to the viscosity just calculated is read from Figure 5.

The experimental work done in the turbulent region was very extensive and reached the point at which the rolling motion of the ball ceased to be uniform. The absolute limit of applicability of the instrument as a viscometer is a Reynolds number of about 800. Since the instrument loses its sensitivity in the turbulent region, its use is not recommended when ratio f/f_c is less than 0.25.

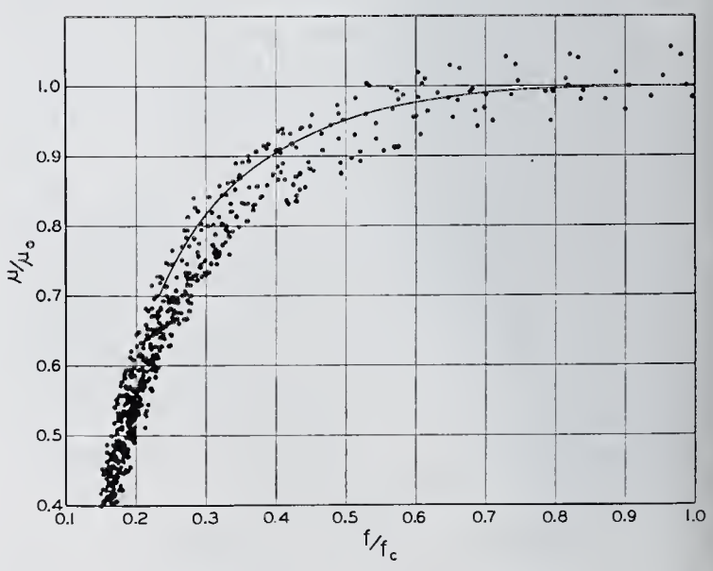

FIGURE 5. CORRECTION FOR ROLLING BALL VISCOMETER IN REGION OF TURBULENT FLOW

TABLE III. CALCULATIONS FROM DATA OF BENNING AND MARKWOOD (2) AND SPÉE (16)

Fluid	Tube Diameter, D	Ball Diameter, d	Diameter Ratio, d/D	Temp.	Fluid Viscosity, μ	Ball Density, ρ_s	Fluid Density, ρ	$\rho_s - \rho$	Driving Force, R	Roll Time	Roll Velocity V	Fluid Velocity, u	Resistance Factor, f	Reynolds Number, Re	Correlation Factor, K
	Cm.	*Cm.*	*Cm.*	*° C.*	*G./cm. sec.*	*G./cc.*	*G./cc.*	*G./cc.*	*G. cm./sec.²*	*Sec.*	*Cm./sec.*	*Cm./sec.*			
Air[a]	1.5934	1.5905	0.9982	1.4	1.715×10^{-4}	2.405	0.0013	2.404	3.492×10^{3}	36.0	0.278	76.0	5.59×10^{7}	1.652	1.084×10^{-8}
				1.7	1.717	2.405	0.0013	2.404	3.492	36.1	0.277	75.8	5.60	1.643	1.087
				30.7	1.859	2.404	0.0012	2.403	3.491	38.3	0.261	71.5	6.98	1.298	1.105
				40.0	1.904	2.403	0.0011	2.402	3.489	39.0	0.256	70.2	7.49	1.205	1.110
				49.7	1.949	2.403	0.0011	2.402	3.489	39.6	0.253	69.2	7.93	1.125	1.121
				79.5	2.086	2.402	0.0010	2.401	3.488	41.6	0.240	65.8	9.59	0.915	1.140
Water[a]	1.5934	1.5805	0.9919	0.5	17.63×10^{-3}	2.403	1.000	1.403	2.00×10^{3}	144.9	0.0690	4.21	67.8×10^{4}	3.08	4.79×10^{-7}
				30.0	8.00	2.402	0.996	1.406	2.005	65.4	0.1530	9.34	13.8	15.00	4.81
				59.6	4.73	2.401	0.983	1.418	2.020	38.5	0.260	15.85	4.92	42.5	4.79
Chloroform[a]	1.5934	1.5805	0.9919	29.67	5.12×10^{-3}	2.402	1.4079	0.9311	1.327×10^{3}	63.4	0.1578	9.61	5.88×10^{4}	35.6	4.78×10^{-7}
				0.46	6.97	2.4026	1.5256	0.8770	1.250	91.1	0.1096	6.69	11.00	18.9	4.81
				29.67	5.12	2.4017	1.4709	0.9308	1.328	63.4	0.1578	9.61	5.88	35.6	4.78
				59.36	3.92	2.4008	1.4142	0.9866	1.406	46.0	0.2174	13.25	3.37	61.6	4.81
				0.46	6.97	2.4026	1.5256	0.8770	1.250	95.1	0.1052	6.41	11.98	18.1	4.61
				30.91	5.07	2.4017	1.4683	0.9334	1.330	64.6	0.1548	9.44	6.11	35.2	4.64
				29.93	5.12	2.4013	1.4702	0.9315	1.328	65.2	0.1534	9.36	6.18	34.7	4.66
Freon-113[a]	1.5934	1.5805	0.9919	0.48	9.28×10^{-3}	2.4026	1.6202	0.7824	1.228×10^{3}	141.5	0.0707	4.32	24.35×10^{4}	9.75	4.21×10^{-7}
				29.88	6.19	2.4017	1.5534	0.8483	1.221	86.9	0.1150	7.02	9.58	22.8	4.58
				0.47	9.28	2.4026	1.6202	0.7824	1.228	141.2	0.0708	4.32	24.35	9.75	4.21
Air[b]	2.012	1.997	0.9925	19.5	1.813×10^{-4}	1.174	0.001	1.173	4.379×10^{3}	...	....	99.1	1.65×10^{6}	9.85	6.2×10^{-8}
	1.281	1.271	0.9922	19.8	1.815	1.1646	0.0012	1.1634	1.122	...	....	471.	4.22×10^{4}	31.0	7.6×10^{-7}
	2.017	1.997	0.9901	19.5	1.813	1.174	0.001	1.173	4.379	...	....	294.3	1.09×10^{5}	37.0	2.5×10^{-7}
Water[b]	1.301	1.271	0.9769	12.0	12.78×10^{-3}	1.1646	0.9995	0.1651	159.0	...	....	4.151	1.03×10^{4}	9.75	1.0×10^{-5}

[a] From Benning and Markwood: tube inclination = 80°; sine θ = 0.9848; roll distance = 10.00 cm.
[b] From Spée: tube inclination = 66°; sine θ = 0.9136.

Effect of Temperature Changes

The effect of temperature changes on the rolling ball viscometer is appreciable when high temperatures are employed or when materials with high coefficients of thermal expansion are used. The effect is greater if the ball and tube are of different material and when the ratio of diameters approaches unity.

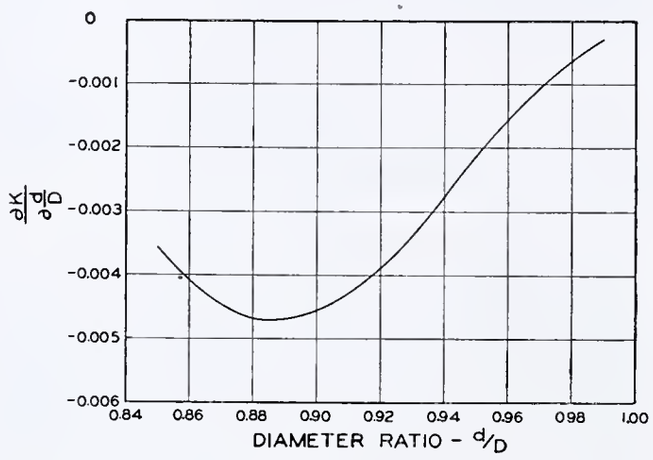

FIGURE 6. RATE OF CHANGE OF CORRELATION FACTOR WITH DIAMETER RATIO

When the rolling ball viscometer is used at a temperature other than that at which it was calibrated, the density of the fluid at the temperature used must be known. The value of factor C of Equation 14 will change as the operating temperature is changed, and the direction and magnitude of the change may be calculated from a knowledge of the average linear coefficients of expansion of the tube and ball materials. The general calibrating equation is written in the form

$$\mu = \frac{5\pi}{42} g \sin\theta \frac{\rho_s - \rho}{V} K\, d(D + d) \tag{16}$$

Temperature changes have an effect only on the terms ρ_s, K, and $d(D + d)$. The coefficient of the calibrating equation must be estimated at each temperature and is the product of the numerical factor and the variable terms of the above equation.

EFFECT ON TUBE. The increase in length and diameter of the tube is calculated from the dimensions and average linear coefficient of expansion of the tube material. The rolling velocity is obviously the calculated length of tube divided by the observed roll time.

EFFECT ON BALL. An increase in temperature increases the diameter and decreases the density of the ball. The change in ball density is given by the relation

$$\Delta\rho_s = \frac{-3\alpha_b\,\Delta t}{1 + 3\alpha_b\,\Delta t}\,\rho_{so} \tag{17}$$

The term $(\rho_s - \rho)$ in Equation 16 is mainly affected by the change in fluid density.

EFFECT ON DIAMETER RATIO. If the ball and tube are made of the same material there is no change in the ratio of diameters and no change in the value of the correlation factor, K, when the temperature is increased. A change in the ratio of diameters causes a change in the value of the correlation factor which depends on the value of d/D and the rate of change of K with d/D. This rate is shown graphically in Figure 6. The change in the diameter ratio with temperature is estimated from the individual coefficients of expansion and the relation shown in Equation 18.

$$\Delta \frac{d}{D} = \frac{(\alpha_b - \alpha_t)\ \Delta t}{1 + \alpha_t\ \Delta t}\left(\frac{d}{D}\right)_o \tag{18}$$

The rate of change of K from Figure 6 is then multiplied by the change in d/D from the equation above to obtain the estimated change in the correlation factor.

Effect on Diameter Product. The linear dimensions of the apparatus combine in the term $d(D + d)$ to affect the calibration when the temperature is changed. A general expression for the change in this term is

$$\Delta[d(D + d)] = [(D_o + 2d_o)\ \alpha_b + D_o\ \alpha_t]\ d_o\ \Delta t \tag{19}$$

If the ball and tube are made of the same material the coefficient of the calibrating equation still changes because of the change in the value of this diameter product.

Comparison with Data in the Literature

Apparently the work of Hoeppler (*10*) was done on apparatus almost identical with the instrument now marketed under his name. Although he failed to give the diameter of tube and balls, other details, including the ratio of the radius of the tube to radius of ball, were given. In describing the commercial instrument Knop (*12*) gave the diameter as 1.5985 cm. and Schrader (*15*) gave 1.5987 cm. Using the data of Hoeppler on air and castor oil, the values of the resistance factor and Reynolds number were calculated for seven ball sizes. The correlation factor, K, was calculated from the single values for each condition. The detailed calculations are given in Table II and the calculated values of K are plotted on Figure 4.

Four out of six values of K calculated from these data check the present work, and the remaining two values are within about 40 per cent of the corresponding values read from the curve. Hoeppler's instrument was almost twice as large in diameter and inclined at an angle of 80° from the horizontal. Since sliding motion of the balls was probably more pronounced with this instrument, the agreement of these data with the present work is unexpectedly good. The values of Reynolds number limiting the streamline region cannot be applied to this instrument.

Benning and Markwood (*2*) used a modified Hoeppler instrument to measure the viscosity of gases and liquids. They derived a calibration equation for the ball and tube combination used on gases from an interpolated value of roll time in air at 20° C. Measurements in air were made at temperatures from 1.4° to 79.5° C., and the viscosity was calculated from the experimental values. The calculated viscosity did not check the critical values (*11*) for air and the deviation from the critical values was greater than the normal dispersion of data for air. Using the original data and the viscosity of air from International Critical Tables, the correlation factor, K, was calculated at seven temperatures. There was a definite trend of the values with changing temperature.

Apparently the ball used in this work was of glass. If it is assumed that the ball and tube material were the same, the diameter ratio and correlation factor would not change with temperature. The increase in diameter product in the temperature range used was small and did not account for the change indicated. It is concluded that the ball and tube were not made of material having the same coefficient of thermal expansion.

The ball and tube combination used on liquids and vapors was calibrated with water and two other known liquids at temperatures from 0° to 60° C. A definite trend in the calculated correlation factor was not evident, but in this case the diameter ratio was smaller and the apparatus was not so sensitive to temperature changes. The calculated values agree well with themselves but are 40 per cent smaller than the values found in the present work. The detailed calculations on the data of Benning and Markwood are given in Table III and the values of the correlation factor calculated from their data are plotted in Figure 4.

Spée (*16*) described experimental measurements of the fluid velocity required to suspend spheres in inclined tapered glass tubes. The full effective force of gravity was used in maintaining the position of the ball against the flowing fluid. In four cases of streamline flow values of the resistance factor, Reynolds number, and correlation factor were calculated. The detailed calculations are given in Table III. The points plotted on Figure 4 agree with the present work in two out of four cases, but the other values are of the right order of magnitude.

Summary

By the use of dimensional analysis, the variables involved in the calibration of the rolling ball viscometer have been combined in the Reynolds number containing the variable viscosity and a resistance factor proportional to the driving force on the ball. A general equation showing the relation between all variables was obtained from a correlation of these factors.

The usual calibration of the instrument in the streamline region of fluid flow was readily obtained from the general equation. The coefficient of this viscometer calibration can now be predicted from the dimensions of the instrument.

When data are taken with the rolling ball viscometer in the turbulent region of flow, the true viscosity can be estimated by applying an empirical correction to the viscosity calculated from the calibration valid only for the streamline region of flow.

A study of the effect of a change in operating temperature on the calibration has been made for the first time. The effect of temperature is appreciable when the coefficients of expansion are different, when temperature changes are large, and when close-fitting balls are used.

Because of the greater sensitivity to viscosity in the streamline region of fluid flow, best results as a viscometer are obtained when the instrument is used in this region. The relations presented have practical value in allowing the viscometer to be designed for a specific purpose or in allowing its range in the more applicable streamline region to be determined. A study of the variables involved will indicate the best design which should result in experimental measurements of greater reliability.

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An Electrically Heated Melting Point Apparatus

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AN ELECTRICALLY heated melting point apparatus with which it is possible to determine the melting point of substances in strict accordance with the heating rates prescribed by the United States Pharmacopoeia (*1*) is described. The relative simplicity and ease of operation make the apparatus very satisfactory for routine laboratory testing where a high degree of reliability is essential.

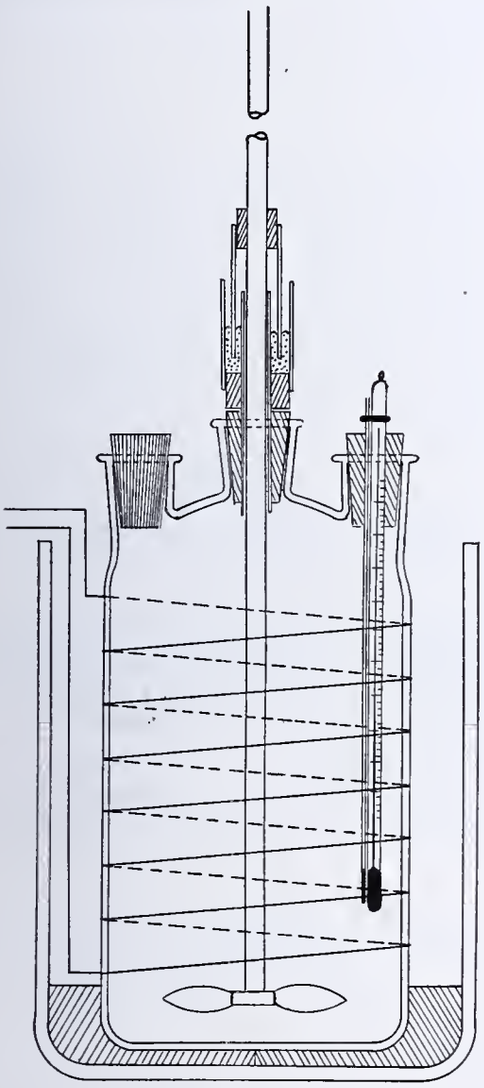

FIGURE 1. MELTING POINT APPARATUS

The volume of the bath used, 450 ml., while appreciably larger than those ordinarily employed for melting point determinations, is of the minimum size consistent with close temperature control. Most textbooks do not sufficiently emphasize the necessity for decreasing the rate of rise in temperature to 0.5° C. per minute at a point 3° or 4° below the supposed melting point of the material being tested. The authors feel that this is a serious oversight, for, unless this is done, high results are almost always obtained.

Because of the nature of the bath used, the apparatus should not be heated above 280° C. If it is to be used at higher temperatures, an adequate quantity of potassium sulfate or other suitable salt should first be dissolved in the sulfuric acid.

Apparatus

A thin-walled, three-necked (16, 19, and 16 mm. in inside diameter) Woulff bottle (Figure 1), approximately 75 mm. in diameter and 130 mm. high (to shoulder), is wound with seven turns of Nichrome ribbon having a resistance of 1.656 ohms per foot (1.5 × 0.125 mm.), the first and last turns being anchored by means of two metal bands, 0.6 mm. thick and 6 mm. wide, passing entirely around the bottle. To prevent the short-circuiting of a portion of the ribbon, a thin sheet of asbestos is interposed between the metal surfaces wherever the wire passes beneath the metal bands. (The bottle is specially blown by The Emil Greiner Co., New York, N. Y. The lower part consists of a 500-cc. tall-form Pyrex beaker. The upper part is made by cutting down a second beaker, to the bottom of which are attached the three necks. The two parts are then fused together.)

The bottle, thus wound, is set into a Pyrex battery jar (standard Pyrex battery jar, 116 × 225 mm. cut down to size) approximately 116 × 140 mm., and is held in place by a layer of asbestos fiber 20 mm. thick having a central depression about 10 mm. deep in which the bottle rests. This asbestos layer may be prepared by moistening long asbestos fiber with water, molding into shape, and then drying slowly at a moderate temperature.

The position of the bottle is so adjusted that the three necks lie in a direct line with the observer. An Anschütz thermometer of appropriate range is placed in the opening nearest the observer and held by means of a molded asbestos stopper. The hole in the stopper is cut slightly elliptical, so that both the thermometer and melting point capillary may be passed through it. A short section of rubber tubing serves to hold the capillary in place as well as to prevent the thermometer from falling into the bottle.

In order to assure atmospheric pressure in the bottle and at the same time prevent the escape of sulfur trioxide fumes due to the gradual decomposition of sulfuric acid at the higher temperatures, a "breather" consisting of a loose roll of thin asbestos sheeting is set in the opening farthest from the observer. A

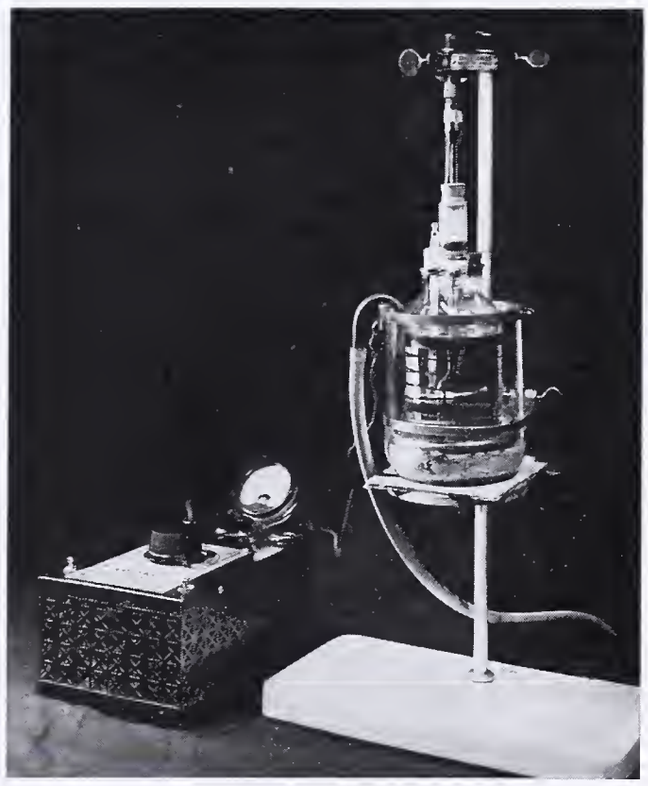

FIGURE 2. MELTING POINT APPARATUS

small motor-driven stirrer, fitted with a mercury seal, completes the setup.

The electrical input to the heating coil is controlled by means of a variable-voltage transformer—similar to the "Voltrol", Type T 1404—having a capacity of at least 6 amperes at 110 volts. The current is measured by an ammeter having a range of 0 to 6 amperes in 0.2-ampere divisions.

After using, the apparatus may be cooled quickly by blowing a current of air through the space between the bottle and the jar. Figure 2 shows the arrangement used by the authors, the air being distributed by means of a perforated brass ring made of 6.25-mm. tubing, set on the asbestos layer.

Calibration

No claim is made for the originality of this method. Credit, however, is given to Leo A. Flexser as the first to employ the method in this laboratory.

Having assembled the apparatus as indicated above, 450 ml. of sulfuric acid (specific gravity 1.84) are introduced and the speed of the stirrer is adjusted to a rate sufficiently high to assure effective distribution of heat. A current is then made to flow through the heating coil by closing the circuit, and is then adjusted to a definite value by applying the necessary potential across the unit by means of a variable voltage transformer. The temperature of the acid is noted periodically by means of a thermometer having a range from 0° to 300° C. In this work the authors found it best to make runs between 1.5 and 6 amperes in 0.5-ampere steps.

The temperatures so recorded are plotted as shown in Figure 3, and the point at which the slope of each curve is 0.5 and 3 (corresponding to a temperature rise of 0.5° and 3° C. per minute) is approximated graphically. This may easily be done by cutting two small right triangles, from a file card or other similar paper, having side ratios of 0.5 and 3. To determine the temperature at which a given amperage will give the desired heating rate, the base of the triangle is set horizontally and the triangle

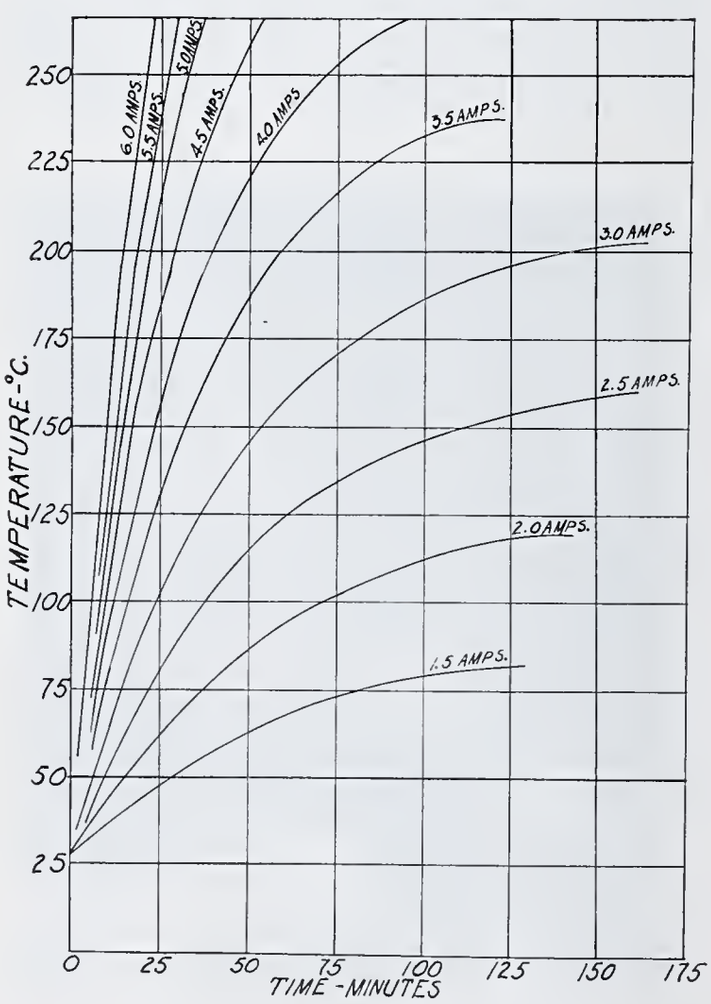

Figure 3. Time-Temperature Curves

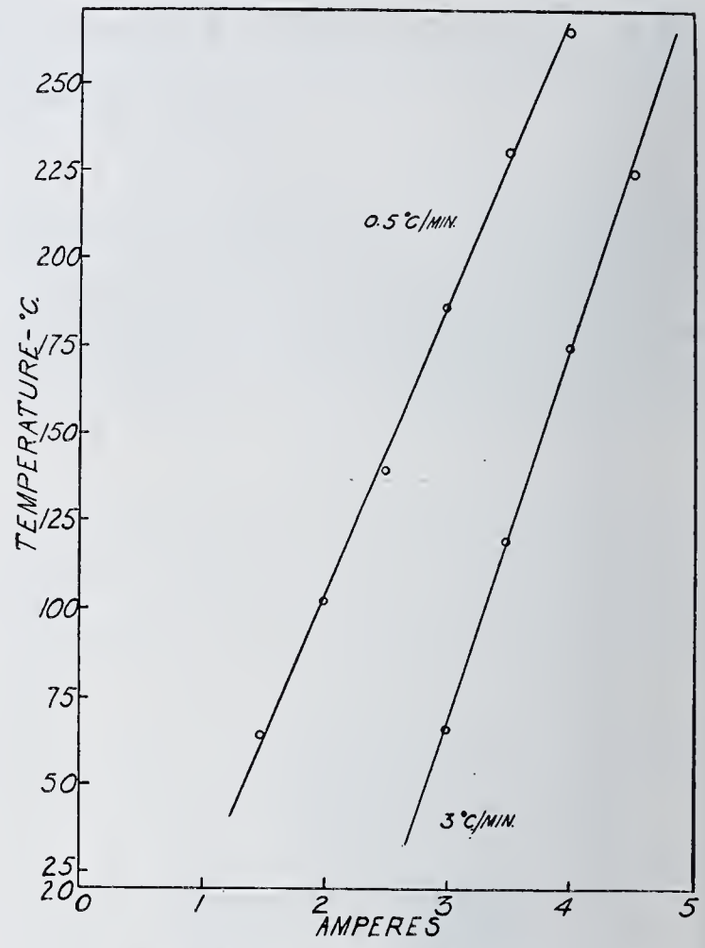

Figure 4. Constant Gradient Curves

is moved along the curve under consideration until a reasonably good fit is found. The temperature line intersecting the midpoint of the hypotenuse of the triangle is the value sought. This procedure is repeated, with both triangles, on all curves. The temperatures so noted are then plotted against the corresponding amperage as shown in Figure 4.

The amperage required to give a temperature rise of 0.5° and 3° C. per minute at various temperatures is then read from the respective curves (Figure 4) and the results are tabulated as shown in Table I.

Table I. Amperes Required to Give Indicated Heating Rate at Various Temperatures

Temperature	Heating Rate 3° C. per minute	Heating Rate 0.5° C. per minute
° C.	*Amperes*	*Amperes*
50	2.8	1.4
60	2.9	1.5
70	3.0	1.6
80	3.1	1.7
90	3.2	1.8
100	3.3	2.0
110	3.4	2.1
120	3.5	2.2
130	3.6	2.3
140	3.7	2.4
150	3.8	2.6
160	3.9	2.7
170	4.0	2.8
180	4.1	2.9
190	4.2	3.1
200	4.3	3.2
210	4.4	3.3
220	4.4	3.4
230	4.5	3.5
240	4.6	3.7
250	4.7	3.8
260	4.8	3.9

Operation

After calibration, the apparatus is ready for use. The speed of the stirrer is adjusted to such a value that thorough mixing of the bath is assured without undue agitation. The capillary tube containing the material, the melting point of which is to be determined, is attached at its uppermost end to an Anschütz thermometer of appropriate range. The thermometer and capillary are then inserted in the asbestos stopper previously described, and put into the bath in such a position that both the material being tested and the thermometer graduations within the suspected melting point range are clearly visible.

The initial current to be used will depend upon the melting point of the substance, as much as 6 amperes being used for the higher temperatures. The temperature of the bath is raised rapidly to a point approximately 30° C. below the supposed melting point of the material. At this point the amperage is reduced to the value required for a temperature rise of 3° per minute. This heating rate is continued until the substance softens. The amperage is then further reduced to the value required for a temperature rise of 0.5° per minute, which rate is maintained until the substance melts.

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Simplified Cell Unit for Internal Electrolysis

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IN ORDER to follow the bismuth content of lead in certain processing operations, it is necessary to have a rapid, accurate method of determination. The oxychloride and other older methods sacrifice accuracy for speed. The internal electrolysis method described by Clarke, Wooten, and Luke (*1*) appeared to hold promise, but because duplication of the apparatus recommended proved difficult, the authors constructed a simplified cell unit from laboratory stock parts. This unit provides greater compactness and convenience of operation at a fraction of the cost of construction. The methods employed were those recommended by Clarke, Wooten, and Luke (*1*).

The design illustrated is assembled entirely from inexpensive stock items. The only section not found in any laboratory stock room is the hard-rubber base plate, which presents no construction difficulties. The air stirrer shown in the illustrations not only is economical, but eliminates a source of accidental error through contamination of the electrolyte during electrolysis, by copper salts from the electric motor. It also permits more compact assembly of the unit.

The cell consists of a hard-rubber or similar electrode support with three hard-rubber binding posts connected together on the upper side with a thin copper strip. Other parts of the cell—flushing tubes, anodes and shells, and the cathode—are attached to the hard-rubber base, which rests on the rim of a beaker during electrolysis with anodes and cathode suspended from it into the electrolyte. At completion of deposition, the unit is disconnected from the anolyte reservoir by one rubber tube connection, and lifted from the electrolyte with simultaneous flushing of the cathode. The continuance of the e. m. f. prevents resolution.

Figure 1 shows details of the anode construction. The flushing tubes serve as the anode cores, and the method by which the anodes are supported by the flushing tubes and rubber tubing above the plate should be noted. The binding posts are attached by tapped holes, but do not continue through to the under side. It is important to have no metals exposed on the under side. This design necessitates bending the cathode stem to avoid a hole through the plate, as shown in Figure 2. Figure 3 shows the relative positions of anodes and cathode and the extension of the cathode position for the introduction of the stirrer through the center.

Figure 4 shows the unit on an ordinary ring stand with all parts in place, exactly as it appears in operation. A small hot plate with high-, medium-, and low-temperature adjustments will be found satisfactory for this purpose. One valve, not two, in the previously designed apparatus is used for anode flushing. The plainly visible outlet tubes make this feature practicable.

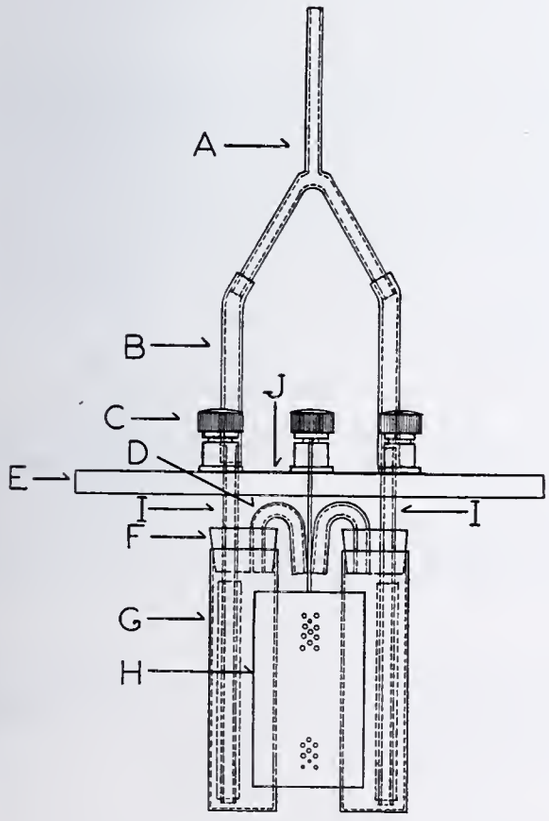


FIGURE 1. ANODE CONSTRUCTION

A. Stock tubing. 0.25 inch in outside diameter
B. Short length of rubber tubing connecting flushing tubes to Y-tube, 6 to 7 cm. long
C. Binding posts, 3; one in center, outer ones 2.5 cm. from center, in line, on either side
D. Overflow tubes, same size as flushing tubes, bent U-shaped, inverted in use. These must turn to drain in opposite direction from cathode. A drop of anolyte might otherwise accidentally fall on cathode during removal (Figures 2 and 3)
E. Hard-rubber, Bakelite, or similar material plate, 13 cm. long, 0.7 to 1.0 cm. thick, 3 cm. wide. Slight variations in dimensions are permissible
F. Rubber stoppers with two holes to accommodate tubing (flushing and overflow tubes), also a very small hole to permit passage of anode leads
G. Alundum shells covering anodes, Norton Co.'s RA360, 2 × 9 cm. These are cut down to 7-cm. length to fit standard 400-ml. beaker
H. Platinum cathode, 5.5 cm. long, 3 cm. in diameter, stem bent as shown in Figure 2. Clearance of cathode and anode shells should be at least 1.5 cm. Cathode must extend beyond edge of hard-rubber base to permit stirrer operation through center (Figure 3)
I. Anode flushing tubes serving also as core for anode wire winding. They extend close to bottom of anode shells, and upwards through hard-rubber plate to 1.5 to 2.0 cm. above upper surface. Lead wire winding begins at extreme lower end of these tubes and continues upward to as close as possible to rubber stopper. Length of tubes will determine clearance of overflow tube from bottom of plate
J. Copper strip, 6.5 × 1.0 cm. connecting bases of all binding posts. After insertion should be greased lightly to minimize corrosion

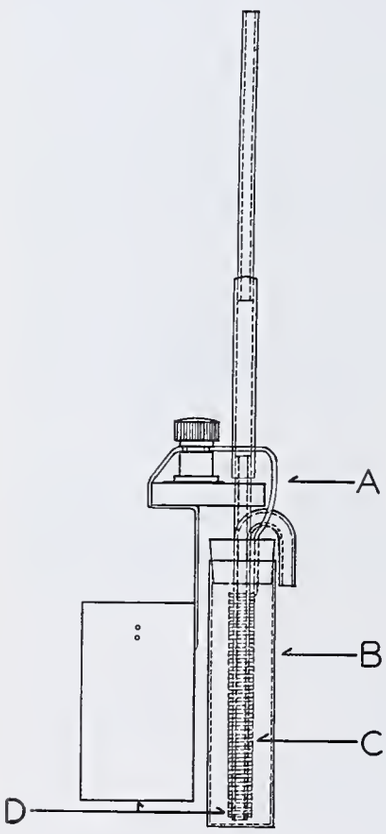
Table I. Determination of Bismuth

Sample No.	Bismuth Found: Internal electrolysis	Bismuth Found: Oxychloride
314	0.083	0.080
316	0.073	0.071
318	0.084	0.082
319	0.036	0.036
320	0.039	0.039
321	0.087	0.084
326	0.129	0.136
H4456	0.022	0.023
H4470[a]	9.20	9.20
352	0.099	0.099
H168	0.018	0.018
H4792	0.019	0.017
H92	0.016	0.016

[a] By aliquoting equivalent of 0.2 gram of sample.

Operation and Care of Cell Unit

As will be seen from Figure 4, a compact series of these units may be easily arranged. The method (*1*) stresses the use of the cell for the determination of small amounts of copper and/or bismuth. However, considerably larger amounts may be safely handled by reducing the weight of the sample used, either by direct weight or by diluting and aliquoting. The limiting factor is the amount of bismuth which will form an adherent deposit. On perforated cathode used, this is approximately 30 mg. Beyond this point difficulty will be experienced in washing and handling because of the powdery characteristics. By starting electrolysis at from 40° to 50° C. and gradually raising the temperature to 70° C., some slight improvement in adherence may be noted when working in the maximum weight range. Considerable improvement in this respect will occur in the presence of copper, which may be added in known amounts for this purpose, if desired. Washing of the cathode during removal from the electrolyte should be done with generous quantities of water, but under a minimum of pressure.



A. Anode lead through small hole in rubber stopper to outside binding posts. Anode wire is high-purity lead of No. 12–16 B. & S. gage
B. Anode shell
C. Flushing tube serving as lead anode wire core
D. Relative positions of lower edge of cathode and anode

Figure 2

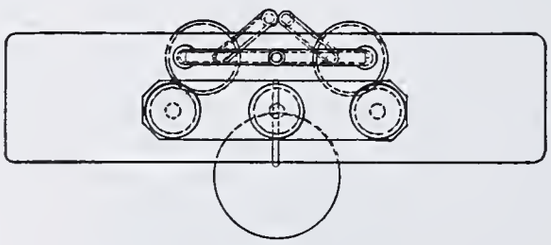


Figure 3. Top View

Shows clearance of cathode and anode shells as well as extension of cathode position to accommodate centrally positioned stirrer. Note location and direction of overflow tubes.

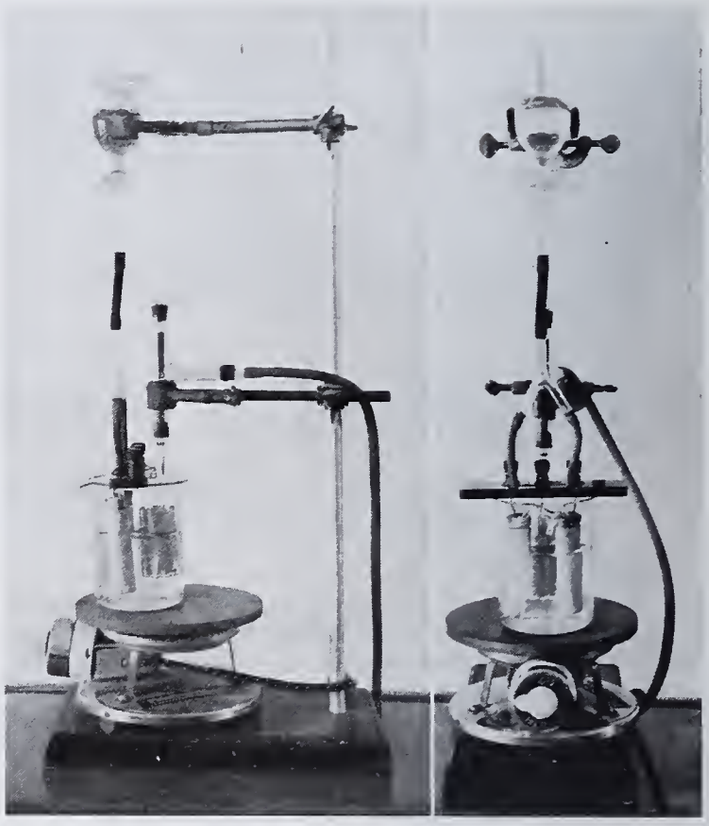


Figure 4. Cell Unit

Care of Alundum Shells (*1*). When disassembling, solution of the pure lead anode wire may be reduced considerably by removing the anode shells immediately after electrolysis and thoroughly rinsing the wire with water. The shells should be immersed in water. When the apparatus has not been used for several hours, wires should be washed off with dilute (10 per cent) nitric acid, followed with water, before each electrolysis. As many as 50 determinations may be made with a single winding, if unnecessary corrosion is avoided. Contacts should be kept efficiently clean and secure at all times. Operation at temperatures above 75° C. should be avoided. Use of thermometer in trial determinations to check temperature controls of the hot plate will be helpful.

Results

A number of comparisons with the oxychloride method are given to demonstrate the accuracy of the internal electrolysis technique (Table I).

These results give ample evidence of the value of the methods of Clarke, Wooten, and Luke (*1*), as well as the practical aspects of the cell unit described here.

The Stirrer

Owing to objections to the electric motor stirrer, an all-glass device has been used. It may be made in the laboratory or purchased from laboratory supply houses.

Literature Cited

(1) Clarke, Wooten, and Luke, Ind. Eng. Chem., Anal. Ed., 8, 411 (1936).

A Continuous Liquid-Liquid Extractor

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LIQUID-liquid extraction can be an extremely tedious and time-consuming operation, particularly when the two liquids tend to emulsify. Most commercial laboratory continuous extractors, though labor-saving, are expensive and very slow in operation. Hossfeld (*1*) describes an apparatus which is a considerable improvement over previous extractors, but which requires a motor and moving glass parts, always a potential source of trouble in careless or inexperienced hands.

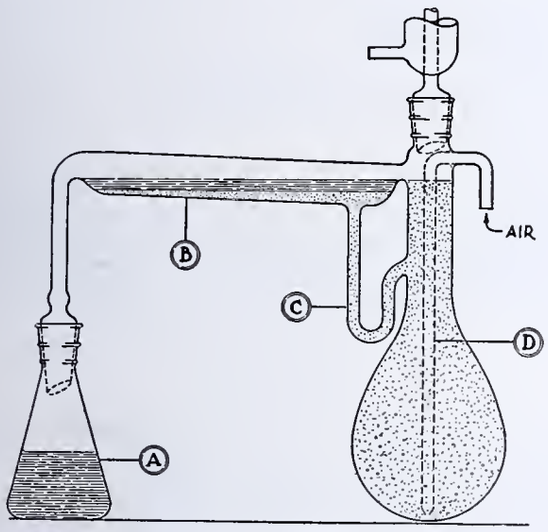

FIGURE 1. EXTRACTOR

The apparatus here described was devised for the routine analysis of cresylic acids, in which pyridine, oils, and naphthalene are extracted with benzene from an alkali solution of the cresylic acid. The potassium phenolates present ordinarily cause a high degree of emulsification. By use of this apparatus, a quantitative extraction is completed in 1 hour, as against more than 12 hours for a commercial extractor dispersing the solvent by means of a fritted-glass bubbler. The apparatus is relatively compact, inexpensive, and simple to build and operate.

The extractor is illustrated in Figure 1. Benzene passes from the boiling flask, *A*, to the reflux condenser, whence it drips into the solution in the mixing flask. Air passing through the inner tube, *D*, enters the flask in a fine jet at the bottom, agitating the mixture vigorously. The mixture of extract and solution overflows into the settling chamber, *B*, from which the extract overflows into the boiling flask, and the solution returns via the trap, *C*, to the mixing flask.

This design represents a simplification of an attempt to eliminate the moving parts in Hossfeld's extractor. The original design is shown in Figure 2. In this case, the reflux was led through inner tube *E* to the intake, *F*, of the aspirator, *G*. The aspirator, operated by a jet of air, circulated fresh solvent through the mixture, while the air served also to agitate the mixture. Surprisingly enough, it was found that removing *E* and closing *F* had no effect on the efficiency of the apparatus. Accordingly, the simpler and more compact device shown in Figure 1 was constructed. In operation, there was no measurable difference in efficiency between these two designs.

There are two possibly undesirable features inherent in the design of the extractor. One is that a long and efficient reflux condenser is necessary to prevent the loss of solvent vapor entrained in the stream of air. However, using a 50-cm. West-type condenser, this loss is negligible in routine work for the 1-hour period of operation. The other disadvantage lies in the fact that the stream of air may oxidize the material being extracted. An inert gas could, of course, be used to obviate this difficulty.

Details of Construction and Operation

The specifications for the extractor are fairly flexible. It was designed to contain approximately 1 liter of liquid, but there is no apparent reason why the flask could not be altered to any desirable shape and capacity. In this case, an 800-ml. Kjeldahl flask with a 24/40 standard-taper neck was used as the mixing flask, and a side arm approximately 25 cm. long was constructed of 3-cm. tubing. The seals at both ends of this arm were made at the top, to allow a maximum amount of space for the separation of the liquids. These openings must be at least 1 cm. in diameter to permit the flow of liquid and vapor in opposite directions. However, the opening at the flask end should not be larger than necessary, lest the turbulence of the contents of the mixing flask be carried into the settling chamber. In order to permit the extracted liquid to flow back to the drain, *C*, the side arm should be sealed on at a slight upward angle. The inner tube, *D*, was a length of 8-mm. tubing, whose lower end was drawn down to an opening of about 0.25 mm. It was sealed through the wall of the flask at the top, to prevent the loss of liquid when the air was turned off.

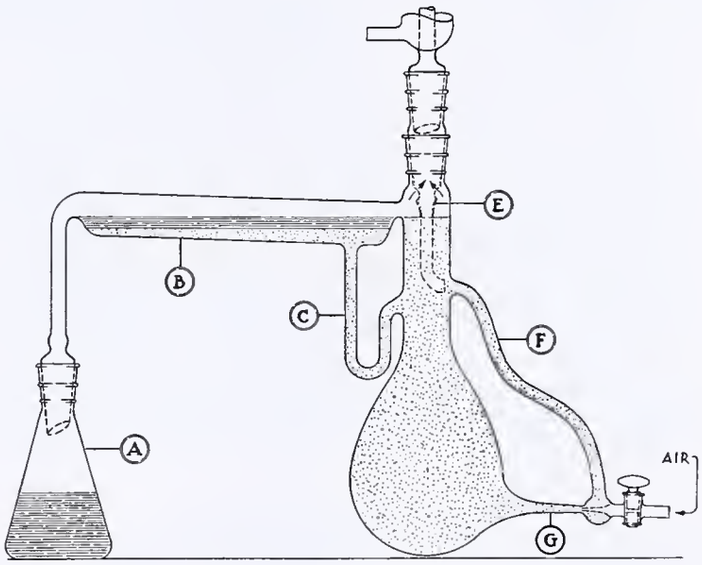

FIGURE 2. ORIGINAL DESIGN

In operation, the flask is filled with the liquid to be extracted to a point just below the bottom of the side arm, *B*. The volume of the air bubbles and solvent brings the level to the overflow. The stream of air should be regulated to the minimum required to give thorough mixing, as indicated by the homogeneity of the contents of the flask. Where colored material is being extracted, completion of extraction is easily estimated by the appearance of the extract in the settling chamber.

Literature Cited

(1) Hossfeld, R. L., IND. ENG. CHEM., ANAL. ED., 14, 118 (1942).

Absorption Tube Tares in Carbon and Hydrogen Microdetermination

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THE microdetermination of carbon and hydrogen as described by Pregl makes use of two absorption tubes, one for water and the other for carbon dioxide. Glass vessels containing lead shot are used as tares. The use of a third absorption tube as a control was described by Friedrich (*2*), who used specially designed tubes that could be closed for weighing. Niederl and Niederl (presumably using Pregl tubes) have suggested the use of a third tube not only as a control but as a tare in order to overcome the effects of high humidity (*3*, p. 114). However, the performance of their absorption system using Pregl type tubes has not been described in the literature. The authors have studied this system extensively and find that its use makes the handling and weighing of the absorption tubes much more flexible. As a result, this part of the carbon and hydrogen procedure is no longer regarded as most subject to error (*3*, p. 123).

Pregl-type tubes are used, since they are the simplest in design and operation. Drierite (20-mesh) is used as water absorbent and Ascarite (20-mesh) as carbon dioxide absorbent. Cotton plugs are used at either end and the stoppered ends are sealed in with Kronig's cement. The tare tube is filled one half with Ascarite and one half with Drierite, with the Ascarite toward the exit end. The weight of the tare tube is adjusted so as to be slightly lighter than the two absorption tubes. When the tubes are put on the combustion line, the tare is also put on the line following the Ascarite tube, and is treated in every way like the absorption tubes. At the balance it is placed on the right-hand pan, while the absorption tubes go as usual on the left. When standing overnight, the tubes are left open to the air on a rack near the combustion apparatus. Dust is kept off by covering the tubes and rack with a folded piece of paper.

When these observations were first begun, the need for the elaborate process of wiping the tubes prescribed by Pregl (*5*) was questioned. It quickly appeared, as Royer (*6*) has also observed, that wiping did not improve the results and often resulted in a drift in the apparent weight, thus requiring a longer waiting period. When the wiping was omitted the tubes became constant within 2 to 3 minutes of putting them on the balance—that is, as soon as the weighing could be completed—and remained constant over a relatively long period of time. The only wiping is given the outside of the tips of the capillaries between the thumb and first finger of the gloved hand, and the inside with a "pipestem" cleaner. A pair of clean white cotton gloves is worn when handling the tubes. The omission of wiping makes possible the use of absorption tubes made of Pyrex despite the statement of Niederl and Niederl (*3*, p. 136).

Table I shows data representing the changes in weight of the absorption tubes over a period of time counting from the moment the tubes were placed on the balance. The first time noted under each experiment represents the time elapsed between placing the tube on the balance and observing its weight.

Discussion

The data of Table I indicate that constant weight is reached about as rapidly as the weights can be added and the weighing operation completed. There is no pronounced tendency for the tubes to change weight, as has been found for other absorption systems (*1*). Thus it is not necessary to have a weighing time schedule. This makes the procedure flexible and therefore advantageous for beginners in the technique, whose working speed may be unusually slow. The greatest change noted in the water tube after 1-hour standing is +0.019 mg. For the four observations, the average change is +0.008 mg. For the carbon dioxide tube the corresponding values are noticeably less. This means that the operation of running carbon and hydrogen

Table I. Constancy of Absorption Tube Weights Using a Third Tube as Control and Tare

Expt.	H_2O Tube Elapsed time	H_2O Tube Change	CO_2 Tube Elapsed time	CO_2 Tube Change
	Min.	*Mg.*	*Min.*	*Mg.*
1	3		3	
	5	−0.002	5	−0.001
	8	−0.006	8	−0.005
			15	−0.008
2	3		3	
	5	−0.001	5	−0.001
	8	−0.003	8	−0.002
3	8		12	
	30	0.006	24	−0.002
4	8		12	
	60	0.019	64	0.000
5	8		12	
	60	0.007	64	−0.004
6	8		12	
	60	0.002	64	0.012
7	8		12	
	60	−0.003	64	0.007
8	8		12	
	90	0.013	94	0.004
9	8		12	
	90	0.024	94	0.014
10	8		12	
	20 hours	−0.020	20 hours	0.010
11	8		12	
	20 hours	−0.038	20 hours	0.015

analyses may, so far as the absorption tubes are concerned, be interrupted without affecting the results. Thus it is not always necessary to have a continuous period of several hours in order to perform carbon and hydrogen analyses. This is of some importance to students whose time is likely to be interrupted. (While such interruptions do not affect the tube weights, some combustion tube fillings, especially those containing lead dioxide, must be conditioned immediately before running an analysis.) The relative constancy of the tubes over a period up to 20 hours indicates the definite absence of any trend in the weight of the tubes. It also indicates that the Pregl tubes may safely be left open to the air for several hours without protecting them with rubber caps.

A great many analyses of research compounds prepared in this laboratory have been run with the absorption system described. The carbon values have agreed regularly within 0.3 per cent relative error and the hydrogen within 1 per cent relative error. However, the high hydrogen values usually associated with high temperatures and humidities (*4*) are still obtained with this system of tares, just as with the lead shot tares, and the proper correction must be determined using a known compound. The high hydrogen values are therefore probably not a direct result of high humidity.

This system of tare requires the use of extra weights from the set up to 300 to 400 mg. to tare the continuous gain in weight of the absorption tubes. The use of so many weights as a tare has not produced a noticeable error in the results.

The elimination of wiping apparently makes the use of Pyrex absorption tubes satisfactory. The omission of the waiting period shortens the total time for the analysis by at least 10 minutes.

Summary

The behavior of a carbon and hydrogen absorption system, in which a third Pregl-type tube is used both as a control and tare, is described.

If the tubes are not wiped, they are constant in weight as soon as they can be weighed. Thus they may be weighed without the usual waiting period.

The tubes remain fairly constant in weight up to periods of 1 hour and show only slight variations over much longer periods. Hence, a rigid weighing program is not required.

The omission of wiping makes possible the use of Pregl tubes made of Pyrex.

The use of a third tube as tare and control does not eliminate the high hydrogen values usually obtained at high atmospheric humidities with other absorption systems.

The use of extra weights to tare the gain in weight of the absorption tubes does not introduce a noticeable error.

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(2) Friedrich, A., *Mikrochemie*, **19**, 23 (1935).
(3) Niederl and Niederl, "Micromethods of Quantitative Organic Analysis", 2nd ed., New York, John Wiley & Sons, 1942.
(4) Power, F. W., Ind. Eng. Chem., Anal. Ed., **11**, 660 (1939).
(5) Pregl, F., "Die quantitativen organische Mikroanalyse", 3rd ed., p. 48, Berlin, Julius Springer, 1930.
(6) Royer, G. L., Norton, A. R., and Sundberg, O. E., Ind. Eng. Chem., Anal. Ed., **12**, 689 (1940).

Microdetermination of Hydroxyl Content of Organic Compounds

Acetic Anhydride–Pyridine Mixture as Reagent

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THE simplest procedures for the determination of the hydroxyl content of organic compounds are those based on esterification. As yet little attention has been given to their application on a micro scale, a field in which they would be especially useful.

Several macro- and semimicromethods (*1*, *2*, *4*, *5*, *7*) employing both acetic anhydride and acetyl chloride have been described in the literature. Extensive esterification experiments with acetyl chloride have been reported recently from this laboratory (*1*). Attempts to use this reagent on a micro scale, however, have led to several other limitations besides those already mentioned (*1*).

Attention was therefore directed to a study of the esterifications with acetic anhydride–pyridine mixture. Both Peterson and West (*4*) and Verley and Bölsing (*7*) have published methods based on the use of this reagent. Stodola (*6*) has reduced the procedure to a micro scale. Since extensive testing of this mixture has not been previously reported, the behavior of a large number of typical alcohols and phenols treated with acetic anhydride–pyridine solutions was studied. As a result of this work a simple microchemical technique based on the use of a hermetically sealed tube has been developed in this laboratory which gives fairly satisfactory results for the microdetermination of the hydroxyl content of organic compounds.

Reagents

c. p. acetic anhydride, redistilled and acetate-free, kept in well-stoppered (screw cap) bottle; c. p. pyridine, redistilled and water-free; and 0.04 *N* sodium hydroxide, carbonate-free.

Apparatus

The reaction vessel consists of a melting point tube, 3 mm. in diameter and 6 cm. in length, made from a soft-glass test tube.

Three medicine droppers, for the delivery of alcohol, acetic anhydride, and pyridine, respectively, are made by drawing one end of a 6-mm. soft-glass tubing to a fine capillary and equipping the other end with a rubber policeman.

Glass plungers, 1.0 mm. × 0.5 cm., are made from soft-glass rod.

A microcentrifuge.

Analytical Procedure

Introduce 2 to 10 mg. of the compound into a weighed reaction tube by means of the dropper, or, in the case of solids, employ the technique described by Niederl for filling Rast tubes (*3*).

Centrifuge and again reweigh the tube. Using the same technique, add approximately 20 to 25 mg. (4 to 5 drops) of pure acetic anhydride from the second dropper, recentrifuge, and weigh

Table I. Acetylation with Acetic Anhydride–Pyridine Mixtures

Primary and Secondary Alcohols	No. of Determinations	OH Theory	OH Found (Average)	Average Deviation
		%	%	*Parts per 1000*
γ-Phenyl-*n*-propyl	2	12.4	12.3	0
Butyl	2	22.9	22.7	5
Isoamyl	2	19.3	19.25	8
Allyl	2	29.3	29.15	2
Cyclohexanol	2	17.0	16.90	6
Octanol-2	2	13.1	12.95	4
Hexanol	2	16.6	15.45	15
Benzyl	2	15.7	15.85	10
sec-Butyl	1	22.9	22.4	..
Cinnamyl	2	12.7	12.25	4
Diethylcarbinol	2	19.3	18.4	5
2-Ethylbutanol	2	16.6	15.9	12
Polyhydric alcohols				
Ethylene glycol	4	54.6	54.0	20
Propylene glycol	6	44.7	41.0	29
Diethylene glycol	4	32.1	30.1	4
Mannitol	2	56.1	56.15	1
Sorbitol	2	56.1	54.7	0
Substituted alcohols				
Ethylene chlorohydrin	4	21.1	20.0	3
1,3-Dichloropropanol-2	2	13.1	12.8	0
Terpenes				
Borneol	2	11.0	11.1	0
Menthol	2	11.0	10.9	9
Geraniol	2	11.0	9.9	0
Eugenol	2	10.4	10.55	5
Isoeugenol	4	10.4	9.75	7
Citronellol	4	10.9	9.45	28
Miscellaneous				
Benzoin	..	8.0	8.11	0
Cholesterol[a]	2[a]	4.4	4.5	11
Phenols				
Phenol	2	18.1	17.65	3
o-Cresol	2	15.7	15.55	10
p-Cresol	2	15.7	15.7	22
m-Cresol	2	15.7	15.35	3
α-Naphthol	2	11.8	11.55	4
β-Naphthol	2	11.8	11.65	4
Resorcinol	2	30.9	30.8	6
Hydroquinone	2	30.9	30.7	3
Orcinol	2	27.4	26.9	4
Catechol	2	30.9	31.05	5
2-Hydroxy-1,4-dimethylbenzene	2	13.9	13.8	8
Pyrogallol	3	40.5	40.0	17
Substituted phenols				
Vanillin	2	11.2	10.85	4
p-Hydroxy-benzaldehyde	2	13.9	13.65	4
Gallic acid	2	27.1	27.3	4
Thymol	2	11.0	11.15	4
Guaiacol	2	13.7	13.65	4
o-Chlorophenol	2	13.2	13.1	0
p-Chlorophenol	2	13.2	13.0	0
Methyl salicylate	2	11.2	11.35	4
Salicylic acid	2	12.3	12.35	4
m-Hydroxybenzoic	2	12.3	12.2	9
p-Hydroxybenzoic	2	12.2	12.25	4
4-Hydroxy-1,2-dimethyl benzene	2	13.9	14.15	4
m-Nitrophenol	2	12.2	12.1	9
p-Nitrophenol	2	12.2	11.35	4
o-Nitrophenol	2	12.2	11.45	2
p-Aminophenol	2	15.6	15.05	1
2-Hydroxy-1,4-dimethylbenzene	2	13.9	13.8	7
Sugars				
Sucrose[b]	4	39.8	39.9	5
Xylose	2	45.5	45.55	3

[a] Hydrolyzed in usual way, then 10 ml. of 95% ethanol added to dissolve ester before titration.
[b] Ran 48 hours.

again. In order to ensure the quantitative conversion of the alcohol to the ester, a ratio of at least 2 moles of anhydride per equivalent of hydroxyl should be maintained.

Add 4 to 6 drops of pure pyridine and again centrifuge. The amount of pyridine does not appear to be critical except in a few cases involving solubility. Insert a small glass rod in the tube, seal, then shake well to ensure complete mixing, and set aside for 24 hours. At the same time run a blank to determine the volume of standard base required to neutralize the acid derived from 1 mg. of acetic anhydride.

Place the reaction tube in a 50-ml. Erlenmeyer flask, add 5 ml. of water, and then break the tube by means of a stout stirring rod. Titrate released acid with 0.04 *N* sodium hydroxide. The per cent hydroxyl can then be calculated by means of the formula:

$$\%\,(\mathrm{OH}) = \frac{(\text{m. e. of anhydride used} - \text{m. e. of acid found}) \times 1700}{\text{mg. of sample}}$$

where m. e. of acid found = ml. × normality, m. e. of anhydride used = mg. of anhydride × ratio × normality, and ratio = ml. of base required to neutralize acid derived from 1 mg. of anhydride.

Results and Discussion

The results obtained with this procedure are given in Table I. In most cases they are the average of duplicate determinations.

This method is not applicable to the determination of tertiary alcohols. Ethyl citrate, *t*-amyl, and *t*-butyl alcohol gave very low results which confirm the observations of others.

Although all experiments were extended over a period of 24 hours at room temperature, these conditions are not to be considered as well-established optima. Since the reaction mixture is hermetically sealed, experiments can be conducted with equal ease at elevated temperatures. Several compounds have been reported to acetylate in 15 minutes at 100° C. in acetic anhydride and pyridine. In this laboratory borneol has been quantitatively acetylated in 35 minutes at 100° C. On the other hand a number of alcohols give poor results at higher temperatures, possibly because of decomposition.

The authors' experience indicates that the ease of acetylation varies with the individual compounds. The ratio of acetic anhydride to alcohol has been found to be somewhat critical and should not fall below 100 mole per cent excess per equivalent of hydroxyl. Since the hydroxyl is determined by difference, it is not a good policy to employ too large an excess for accurate work.

Care should be exercised in the selection of acetic anhydride. It is essential that pure redistilled anhydride (from a good fractionating column) be used to obtain the best results. In some of the initial work erratic results were traced to the presence of a considerable quantity of acetic acid in the acetic anhydride. As soon as this was remedied the results were nearer the theoretical values and were much more reproducible. In later experiments the acetic anhydride was redistilled in a 7-plate column. The titer of this anhydride agreed perfectly with the theoretical value.

The experiments with sugars were performed merely to ascertain the possibilities of a micromethod in this field. As indicated, the results are satisfactory and are in agreement with the data of Peterson and West (*4*).

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Qualitative Analysis of Microgram Samples

Separation, Estimation, and Identification of the More Common Ions of the Hydrogen Sulfide Group

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The technique of working in the capillary cone has been applied to analyses requiring lengthy separations followed by sedimetric estimations and confirmatory tests. Various improvements have been made in the performance of manipulations, and a method for fractional distillation has been developed, which may be applied to liquids of approximately 0.1- to 0.01-cu. mm. volume.

The complete analysis of a particle of Wood's alloy of 1-microgram mass required approximately 12 hours. Such efficiency can be expected only after a period of training. Manual dexterity is perhaps of less importance than methodical organization of work. Proper design of work bench and the use of microprojection will considerably reduce fatigue, if work of this kind has to be performed for any length of time.

THE technique described in preceding papers of this series (*1, 3*) was tested by application to the analysis of rather complex mixtures containing ions of the hydrogen sulfide group. To make the test more severe a scheme of macroanalysis was chosen, which emphasizes quantitative separations and estimation of the amount of the ions.

Technique

The experience gained led to various improvements and extensions of the techniques employed in the transfer of solutions to capillary cones and in the mixing, stirring, and heating of the contents of such cones. These, as well as the procedure for distillation from one capillary cone to another, have been described in detail (*2*). Some advice may be added.

TRANSFER OF SOLIDS TO CAPILLARY CONES. When a sample is selected for analysis, it is desirable to have the material spread for microscopic inspection. The procedure described for the transfer of solid reagents (*2*) has the disadvantage that the use of an inclined slide does not permit a sharp focus for the entire field of vision. If the microscope slide with the material is placed level on top of the chamber containing the capillary cone, a suitable needle for the transfer of particles is obtained by sealing the tip of a micropipet and bending it through an angle of 30 degrees (*4, 9*). The shank of the pipet is then inserted into the holder, so that the tip points downward. When the selected particle has been picked up with the needle, the clamp of the manipulator is released for a moment, and the pipet holder is rotated to bring the tip of the needle into a horizontal plane. It is then easy to introduce the particle into the capillary cone. The procedure is illustrated by Figure 1. Filings of Wood's alloy (specific gravity 7) are first shown on a microscope slide. An approximately spherical particle, 66μ in diameter, which may be expected to have a mass of 1 microgram, has been brought into the center of the field and is just being touched with the point of the needle. On the right is the same particle just before it is deposited inside the capillary cone.

ESTIMATION OF THE QUANTITY OF PRECIPITATES. Figure 2 (left) shows a sulfide precipitate containing 0.1 microgram of antimony and 0.01 microgram of bismuth. Figure 2 (right) shows the same precipitate after whirling in the centrifuge. Obviously, it is collected in the point of the cone, but inspection under higher magnification would conclusively demonstrate that the point of the taper is free from precipitate. Neither base nor top of the truncated cone filled by the precipitate presents a plane surface. Since some arbitrary lines must be drawn when the calculation of the volume of precipitate is based on the assumption of a truncated cone, it seemed well to imagine a sphere containing the precipitate, and to base the estimation on the diameter of this imaginary sphere (*1*). It is understood that a definite amount of centrifugal force was applied for a certain time when collecting precipitates for the purpose of estimation. As a rule, 1 minute of centrifuging with a relative centrifugal force equal to 500 times gravity gave satisfactory results.

USE OF TEST PAPER. Spot tests on paper may be performed in a very simple manner. The paper is cut into strips approximately 3 mm. wide and 20 mm. long. Whenever needed, a strip is placed on the carrier so that it lies flat and parallel to the capillary cones and reagent cones. Approximately one third of the strip projects beyond the edge of the carrier, and this part of the paper is bent downward, so that the opening of the micropipet makes contact with the paper. Test solutions and reagent solutions are transferred from capillary cones or reagent containers to the paper by means of the micropipet. The colorations produced are best observed with low magnification and reflected light of low intensity.

The technique was employed when testing the acidity of solutions with litmus paper and in the confirmatory tests for cadmium (cadion) and tin (molybdenum blue).

PROJECTION OF MICROSCOPIC IMAGES. The operations performed under the microscope are just as easily controlled by observation of a projected image as by viewing through the eyepiece. The glare sent out by the illuminated apparatus on the stage is not very bothersome when the intensity of the light is decreased to that required for a screen image of 10- to 20-cm. diameter. Under this condition, the use of projection will reduce eyestrain, and the operator will be able to assume a more restful posture.

Scheme of Analysis

The scheme of Swift (*12*) was adopted with some modifications. The return, in a sense, of the mercury to the copper

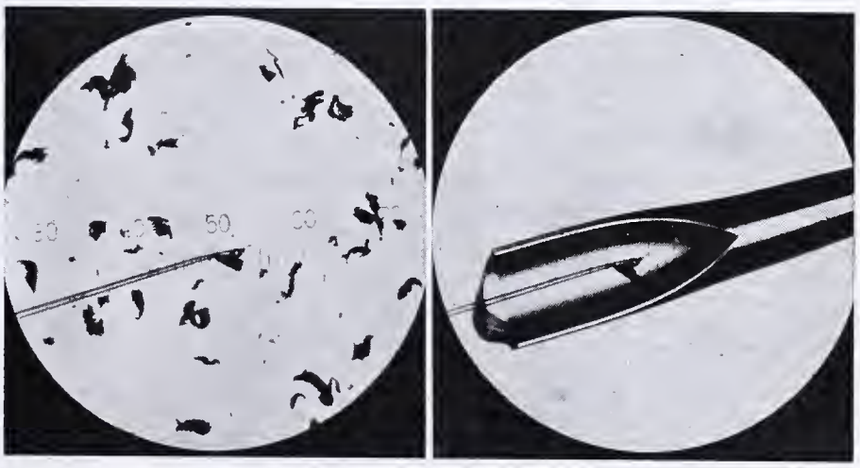

FIGURE 1. TRANSFER OF A PARTICLE OF WOOD'S ALLOY OF APPROXIMATELY 1-MICROGRAM MASS FROM A SLIDE TO CAPILLARY CONE
One division of micrometer scale corresponds to 22 microns. Magnification, × 46

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group probably is the most pronounced deviation, and it was introduced when it was found (*5, 10*) that the extraction of mercuric sulfide from the sulfide precipitate remained incomplete when medium to large quantities of mercury were present. Various minor changes had to be made because of the substitution of sedimetric estimations followed by confirmatory tests for the titrimetric determinations employed by Swift.

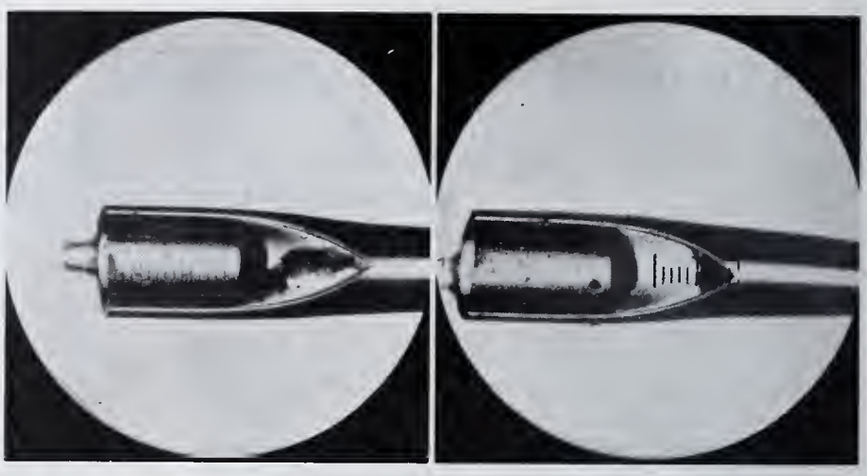

FIGURE 2. SULFIDE PRECIPITATE CONTAINING 0.1 MICROGRAM OF ANTIMONY AND 0.01 MICROGRAM OF BISMUTH

One division of eyepiece micrometer is equal to 21 microns. Magnification, × 48
Left. Contents of capillary cone after treatment with hydrogen sulfide
Right. Precipitate collected in point of cone by means of centrifuge. Estimated that precipitate would fill a sphere of 63-micron diameter, 3 divisions of the scale

PREPARATION OF SOLUTION. In the analysis of Wood's alloy a particle of approximately 1 microgram mass was treated in the capillary cone with 0.01 cu. mm. of 16 *M* nitric acid. Reaction with evolution of gas and separation of a white precipitate started immediately. The mixture of solution and precipitate was evaporated almost to dryness by heating on the steam bath, and the residue was taken up with 0.007 cu. mm. of 12 *M* hydrochloric acid. Most of the residue dissolved, and the mixture was treated with 0.1 cu. mm. of water previous to saturating with hydrogen sulfide.

Known solutions were prepared on a large scale and made approximately 4 *M* with respect to acid. Of these solutions 0.01 cu. mm. was taken for analysis, transferred to the capillary cone, and diluted with 0.1 cu. mm. of water. Precipitates separating because of hydrolysis appear brown in transmitted light and white in reflected light. They were left in suspension for the treatment with hydrogen sulfide.

PRECIPITATION OF THE HYDROGEN SULFIDE GROUP. The solution or mixture of solution and precipitate was saturated with hydrogen sulfide, as described in an earlier communication (*1*). The reaction mixture was heated at 60° to 70° C. for half a minute and then allowed to stand for 1 hour at room temperature. The precipitate was collected by means of the centrifuge and its volume was estimated. Another portion of 0.1 cu. mm. of water was added, and saturation with hydrogen sulfide, heating, standing, centrifuging, and estimation of volume were repeated. The solution was removed, and the precipitate was washed once with 0.01 cu. mm. of 0.12 *M* nitric acid.

SEPARATION OF THE COPPER AND ARSENIC GROUPS. The sulfide precipitate was immediately treated with 0.02 cu. mm. of sodium sulfide–hydroxide reagent (*8*, 48 grams of sodium sulfide novahydrate and 4 grams of sodium hydroxide in 100 ml. of solution). The capillary cone was sealed into a capillary and inserted in a water bath at 60° to 80° C. for one minute. The capillary was withdrawn from the bath a few times, and its contents were stirred by means of the buzzer (*2*). The extraction was repeated with a 0.02-cu. mm. portion of a 1 to 1 dilution of the reagent. The volume of the residual sulfides was estimated before removal of the second extract. The extracts were combined, and the residue was washed with 0.01 cu. mm. of water by stirring and centrifuging.

DISSOLVING THE SULFIDES OF THE COPPER GROUP. The residue from the extraction with sodium sulfide was immediately treated with 0.02 cu. mm. of 3 *M* nitric acid, sealed into a capillary, and heated in steam until solution of the sulfides appeared complete when observed under the microscope. A small residue of white sulfur was collected by means of the centrifuge, and the clear solution was transferred to another cone.

If the precipitate did not dissolve completely, 0.01 cu. mm. more of the 3 *M* acid was added and the heating was repeated. Mercuric sulfide may remain behind with the sulfur. Its volume may be estimated immediately or after solution in nitric-hydrochloric acid and reprecipitation with hydrogen sulfide. Obviously, the solution in nitric-hydrochloric acid may be set aside and later used for dissolving any mercuric sulfide separating from the extract containing the arsenic group.

ISOLATION OF MERCURY. The cone with the sodium sulfide extract was transferred to a dry chamber with a large drop of concentrated ammonia on the bottom plate and then treated with 0.03 cu. mm. of 3 *M* ammonium acetate. The mixture was stirred by means of the buzzer (*2*). The precipitate was collected by whirling in the centrifuge. Particles adhering to the walls of the capillary cone were loosened with the micropipet. The volume of the precipitate was estimated, and the supernatant solution was immediately transferred to another cone. All these operations were performed without delay, for evaporation of ammonia from the capillary cone may lead to separation of the sulfides of arsenic, antimony, and tin from the solution of their thio complexes.

Confirmatory Test for Mercury. The precipitate of mercuric sulfide was washed once with 0.01 cu. mm. of water, then treated with 0.01 cu. mm. of nitric-hydrochloric acid (1 to 1). The mixture was evaporated to dryness on the steam bath. The residue was either dissolved in 0.01 cu. mm. of 0.12 *M* nitric acid and the solution used for the diphenylcarbazide test (*3*), or it was dissolved in 0.005 cu. mm. of water and treated with 0.01 cu. mm. of 1 per cent stannous chloride. The whitish gray precipitate produced by the latter reagent is easily observed with reflected light after collection in the point of the capillary cone by means of the centrifuge.

PRECIPITATION OF THE ARSENIC GROUP. The centrifugate from the mercuric sulfide precipitate, which may contain thio complexes of arsenic, antimony, and tin, was treated with 1 *M* sulfuric acid. Approximately 0.2 cu. mm. of this acid are required, but the relatively large volume is of little consequence. Stronger acid reacts so violently that some of the reacting matter may be ejected from the cone as a consequence of the rapid liberation of hydrogen sulfide.

The sulfuric acid was added in small portions while stirring with the micropipet until the mixture became just acid. For testing, small portions of the solution were transferred to litmus paper. Flocculation of the precipitate was finally made complete by heating in a sealed capillary for a few minutes at 80° to 90° C. The mixture was centrifuged and the solution removed and rejected.

If there is reason to believe that too much acid had been added, one may test for absence of tin from the centrifugate by diluting it with 0.05 cu. mm. of water, saturating with hydrogen sulfide, heating, and stirring by means of the buzzer. An estimation of the volume of the sulfides of the arsenic group requires subtraction of the volume of the sulfur contained in the precipitate. The volume of the sulfur left behind when the sulfides are dissolved allows a crude approximation.

ISOLATION OF ARSENIC. The sulfides of the arsenic group were treated with 0.03 cu. mm. of 12 *M* hydrochloric acid. The mixture was warmed for 15 seconds in a water bath and then thoroughly agitated by means of the buzzer. The capillary cone was then returned to the dry chamber, and its contents were inspected under the microscope. If any sulfide was discovered, the mixture was treated with potassium bromate. One particle of the potassium bromate was added at a time, whereupon the solution was stirred. The addition of bromate was stopped as soon as all the sulfide was dissolved. The sulfur was then collected by means of the centrifuge, and the clear solution was transferred to another capillary cone. The residue of sulfur was washed with 0.03 cu. mm. of 12 *M* hydrochloric acid, and this acid was then added to the solution of the sulfides.

The solution of the sulfides was treated with 0.01 cu. mm. of 9 *M* hydrochloric acid and 0.02 cu. mm. of 3 *M* phosphorous acid. The cone was sealed into a capillary, immersed for 5 to 10 seconds in water at 80° to 90° C., and cooled in a stream of tap water. After insertion of the capillary cone into a distilling capillary (*2*), the distillation was started (Figure 3), and continued until the volume of the residue in the capillary cone was reduced to 0.015 or 0.01 cu. mm. The residue of the distillation was treated with 0.01 cu. mm. of 12 *M* hydrochloric acid, and the

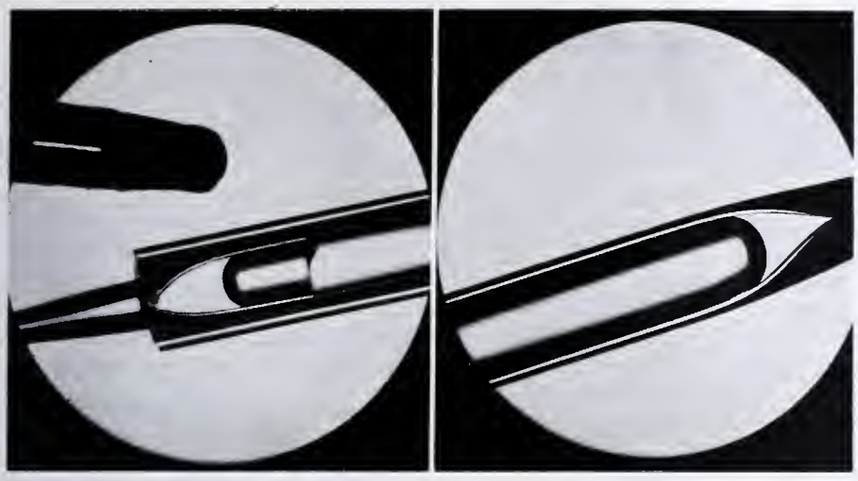

FIGURE 3. DISTILLATION FROM ONE CAPILLARY CONE TO ANOTHER

Photomicrographs show two halves of same distilling capillary. Magnification, × 30
Left. Start of distillation. Heating element is visible above opening of distilling capillary containing capillary cone with liquid to be distilled
Right. Close of distillation. Distillate collected at sealed end of distilling capillary

distillation was repeated until again a residue of the above indicated volume remained behind.

The distillate (Figure 3, right) was transferred to a capillary cone of the standard type, diluted with water to a volume of approximately 0.1 cu. mm., treated with 0.04 cu. mm. of 12 *M* hydrochloric acid, and saturated with hydrogen sulfide. Flocculation of the precipitate was brought about by heating for 30 to 45 seconds at 60° to 70° C. and agitation by means of the buzzer. The volume of the precipitate was estimated after centrifuging.

Confirmatory Test for Arsenic. The supernatant solution was removed, and the yellow precipitate of arsenic sulfide was washed once with approximately 0.03 cu. mm. of water and then dissolved in 0.01 cu. mm. of 6 *M* ammonia. The solution was evaporated almost to dryness by heating in steam, the residue was treated with approximately 0.05 cu. mm. of 16 *M* nitric acid, and the mixture was evaporated to dryness. The residue was extracted with a volume of 1 *M* nitric acid to give an approximately 1 per cent solution of arsenic. Part of this solution was treated on the plateau of the condenser rod with silver nitrate in acetate buffer solution (*3*).

ISOLATION OF ANTIMONY. The residue from the distillation was treated with 0.02 cu. mm. of 3 *M* sulfuric acid and 0.01 cu. mm. of 6 *M* hydrochloric acid, and water was added to bring the total volume to 0.075 cu. mm. Finally 5 cu. mm. of phosphoric acid, obtained by mixing 1 volume of sirupy acid with 4 volumes of water, were added, and the antimony sulfide was precipitated by saturating with hydrogen sulfide, and heating at 60° to 70° C. as described. The precipitate was collected by means of the centrifuge, and its volume was estimated. The solution containing the tin was transferred to another capillary cone. The precipitate was washed with 0.01 cu. mm. of 1.2 *M* hydrochloric acid, which was then combined with the centrifugate.

Confirmatory Test for Antimony. The antimony sulfide was dissolved by heating with 0.02 cu. mm. of 12 *M* hydrochloric acid. Part of the clear solution was transferred to the plateau of the condenser rod and tested with the quinine reagent (*3*).

ISOLATION OF TIN. The centrifugate containing the tin was treated by the addition of small portions of concentrated ammonia until test with litmus paper indicated alkaline reaction. The solution was then saturated with hydrogen sulfide and briefly heated at 60° to 70° C. After the capillary cone had been placed in a dry chamber, 0.005 cu. mm. of 3 *M* sulfuric acid was added to the contents. The precipitate of tin sulfide formed slowly. It could not be readily seen in transmitted light, but was visible in reflected light. Stirring with the pipet hastened flocculation. Precipitate and solution were again saturated with hydrogen sulfide, and the mixture was allowed to stand for at least a few hours. It was then centrifuged, and the volume of the stannic sulfide was estimated.

Confirmatory Test for Tin. The centrifugate was removed from the yellow stannic sulfide, the precipitate was washed once with 0.015 cu. mm. of 1 *M* ammonium chloride, and the sulfide was dissolved by heating in steam with 0.02 cu. mm. of 6 *M* hydrochloric acid. After addition of 0.01 cu. mm. of 12 *M* hydrochloric acid and centrifuging, parts of the clear solution were used for confirmatory tests. Since the crystals of the cesium chlorostannate are very small and difficult to identify, performance of a spot test was preferred. A small particle of magnesium metal, obtained by scraping with a razor on the edge of magnesium ribbon, was introduced into the acid solution. A small portion of the reduced solution was immediately transferred to a strip of ammonium phosphomolybdate test paper (*7*). The blue spot was observed with the use of the general room illumination and a magnification of 28 diameters.

ISOLATION AND CONFIRMATION OF LEAD. The nitric acid solution of the sulfides of the copper group was evaporated to dryness on the steam bath, the residue was treated with 0.015 cu. mm. of 6 *M* sulfuric acid, and the volume of the lead sulfate was estimated. The solution was transferred to another centrifuge cone, and the precipitate was washed once with 0.005 cu. mm. of 0.6 *M* sulfuric acid, which was then added to the centrifugate containing bismuth, copper, and cadmium.

The lead sulfate was dissolved by adding 0.02 cu. mm. of 3 *M* ammonium acetate and stirring. To the solution was added 3 *M* sodium chromate in small portions until yellow coloration of the solution indicated an excess of the reagent. The lead chromate was collected by means of the centrifuge, and its volume was estimated.

ISOLATION OF BISMUTH. The capillary cone with the centrifugate from the lead sulfate was transferred to a dry chamber, and the solution was treated with small portions of concentrated ammonia until a white precipitate could be perceived with reflected light. Then 0.005 cu. mm. more of the ammonia was added, and the mixture was heated for 15 seconds at 90° C. after being sealed into a capillary. After centrifuging, the capillary cone was transferred to a moist chamber for the determination of the volume of bismuth hydroxide.

Because of the transparent appearance of the precipitate, the removal of the supernatant solution and washing of the precipitate with 0.01 cu. mm. of 1 *M* ammonia were observed with reflected light. The washing was combined with the centrifugate containing copper and cadmium.

Confirmatory Test for Bismuth. The bismuth hydroxide was dissolved in 0.005 cu. mm. of 3 *M* hydrochloric acid. Parts of the solution were used for performance of the cesium and quinine iodobismuthite tests (*3*) on the plateau of the condenser rod.

ISOLATION OF COPPER. The copper may be separated from cadmium by precipitation as cuprous thiocyanate. This method was successfully applied to the analysis of centigram and milligram samples (*5*, *10*). In the experiments on the microgram scale copper never occurred with cadmium, and it could be isolated as the sulfide. The volume of the sulfide precipitate served for estimation of the quantity of copper.

Confirmatory Test for Copper. The copper sulfide was treated with 0.02 cu. mm. of 3 *M* nitric acid, the mixture was evaporated to dryness, and the residue was stirred with 0.015 cu. mm. of water. After centrifuging, half of the clear solution was transferred to the plateau of a condenser rod and tested with salicylaldoxime (*3*). The rest of the solution was treated on a plateau with a somewhat larger volume of a saturated solution of potassium ferrocyanide. The brown precipitate of copper ferrocyanide was observed with a magnification of 60 diameters.

ISOLATION OF CADMIUM. The ammoniacal centrifugate from the bismuth hydroxide was treated with 0.005 cu. mm. of 10 per cent potassium cyanide. The clear solution was then saturated with hydrogen sulfide, heated at 65° C. for about 20 seconds, agitated with the buzzer, and centrifuged. The volume of the yellow cadmium sulfide was determined.

Confirmatory Test for Cadmium. After removal of the centrifugate the cadmium sulfide was washed once with 0.02 cu. mm. of water and then dissolved in 0.015 cu. mm. of 6 *M* sulfuric acid. The mixture was briefly heated on the steam bath. After centrifuging, the clear solution was transferred to another capillary cone and treated with an approximately equal volume of buffer solution containing 10 grams of Rochelle salt and 0.1 ml. of glacial acetic acid in 100 ml. Homogeneity was obtained by stirring with the pipet. Approximately 0.015 cu. mm. of the solution was transferred to a strip of paper treated with Cadion 3B reagent (*2*, *6*). After the solution had evaporated on the paper, 0.01 cu. mm. of a solution consisting of 4 volumes of 2 *M* sodium hydroxide and 1 volume of ethyl alcohol was added. The pink coloration produced by the lake of the dye with the cadmium hydroxide was distinctly visible with reflected light.

Results

Application of the technique and scheme of analysis to samples of approximately 1-microgram mass revealed the qualitative composition in each instance. The reliability of the estimation of quantities may be derived from the following three examples.

In 0.01 cu. mm. of a solution containing 0.1 microgram each of the ions copper, arsenic, antimony, and tin were found: 0.1 microgram of copper, 0.06 microgram of arsenic, 0.1 microgram of antimony, and 0.24 microgram of tin.

In 0.01 cu. mm. of a solution containing 0.1 microgram each of mercuric mercury, lead, bismuth, cadmium, and antimony were found: 0.1 microgram each of mercury, lead, bismuth, and antimony, and 0.8 microgram of cadmium.

In 1 microgram of Wood's alloy containing 0.5 microgram of bismuth, 0.2 microgram of lead, 0.125 microgram of tin, and 0.125 microgram of cadmium were found: 10 microgram of bismuth, 0.4 microgram of lead, 0.1 microgram of tin, and 0.1 microgram of cadmium.

The most glaring mistakes were committed in the estimations of bismuth and cadmium, and in both instances the reason must be sought in the poor reproducibility of the volumes of gelatinous precipitates of bismuth hydroxide and cadmium sulfide. The trouble might be overcome by converting the bismuth hydroxide to metallic bismuth and the cadmium sulfide to cadmium-thiourea Reineckate (*11*). The determination of the volume and consequently the mass of the alloy may easily be affected with an uncertainty of ±50 per cent.

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Presented in part before the Division of Microchemistry at the 100th Meeting of the American Chemical Society, Detroit, Mich. Abstracted from part of the thesis submitted by Michael Cefola to the faculty of the Graduate School of New York University in partial fulfillment of the requirements for the degree of doctor of philosophy.

Report on Recommended Specifications for Microchemical Apparatus

SULFUR AND HALOGENS

G. L. ROYER, *Chairman*
H. K. ALBER
L. T. HALLETT
J. A. KUCK, *Secretary*

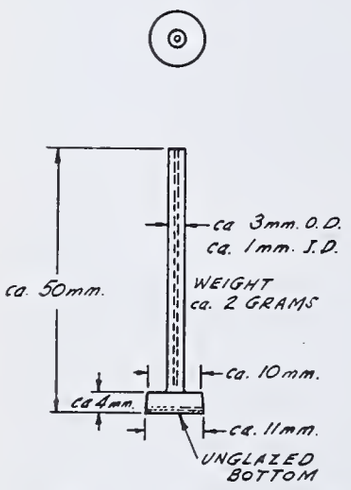

Figure 1. Porcelain Filter Stick

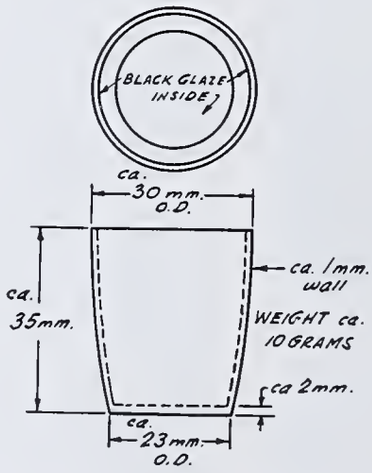

Figure 2. Porcelain Crucible

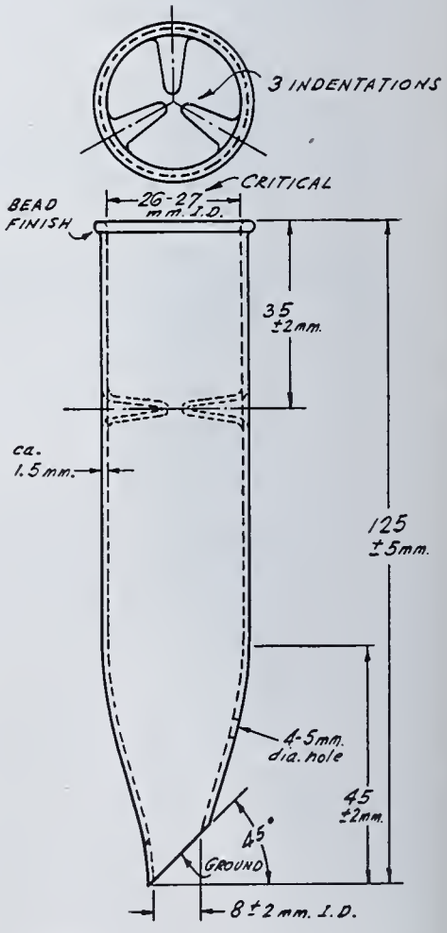

Figure 3. Glass Crucible Holder for Water Bath

A PREVIOUS report covering the recommended specifications for the apparatus for the determination of carbon-hydrogen and Dumas nitrogen (*1*) states the purpose and aims of the work and gives the bibliography from which much of the information was obtained. The present report is a continuation of the same work with regard to the apparatus for microchemical analysis. This present report was approved by the members of the Division of Analytical and Micro Chemistry after its presentation at Buffalo in September, 1942. It has also been approved by a special committee of the Scientific Apparatus Makers of America, appointed by John M. Roberts, president, which consisted of J. J. Moran, chairman, A. A. Kelm, L. D. Wilson, W. B. Warren, and J. E. Patterson. The specifications reported here, having been approved by this committee, have thus received the approval of the Scientific Apparatus Makers. With the acceptance of these recommended specifications by this organization and with the approval of the Division of Analytical and Micro Chemistry, the Committee on Standard Apparatus of the American Chemical Society advocated their publication.

An examination of the drawings shows that certain of the dimensions are theoretically and experimentally critical, while others could be varied over wide limits. However, many of these dimensions, which may not in themselves be critical, can be accurately controlled in the manufacturing process without inconvenience. For this reason, all the dimensions on the drawings are given with specified limits which have been agreed upon by both the manufacturers and the microchemists. We have been unable to describe specifically certain factors, such as the porosity of the sintered porcelain and glass apparatus. Therefore, we must rely upon the manufacturers to control these within the limits necessary to be satisfactory.

The drawings which accompany this report are not reproduced here in full size. Copies of the full-size drawings may be obtained at a nominal cost from the office of the Scientific Apparatus Makers of America, 20 North Wacker Drive, Chicago, Ill.

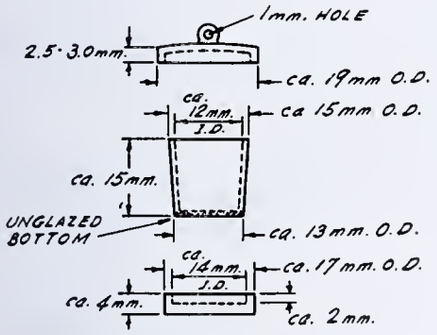

Figure 4. Porcelain Filter Crucible and Ignition Dish

Part III. Sulfur

The following units were considered by the committee as requiring specifications:

- Filter stick (Figure 1)
- Crucible (Figure 2)
- Glass crucible holder (Figure 3)
- Filter crucible and ignition dish (Figure 4)
- Crucible filter assembly (Figure 5)
- Siphon, receiver, and inner container for barium sulfate filtration (Figure 6)
- Glass dome and metal crucible desiccator (Figure 7)
- Micro evaporating dish for sulfur bomb determination (Figure 8)
- Platinum microware for both halogen and sulfur determinations (Figure 9)

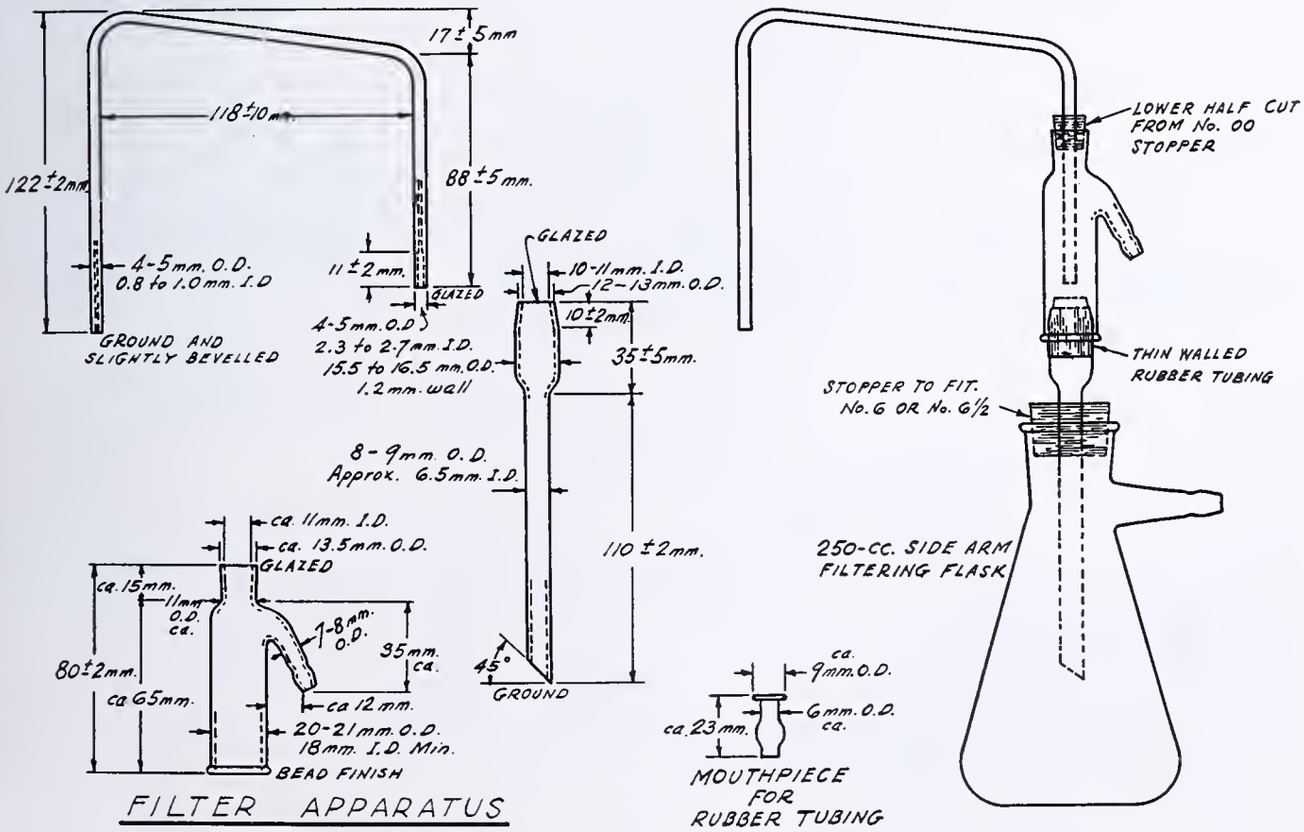

Figure 5. Crucible Filter Assembly

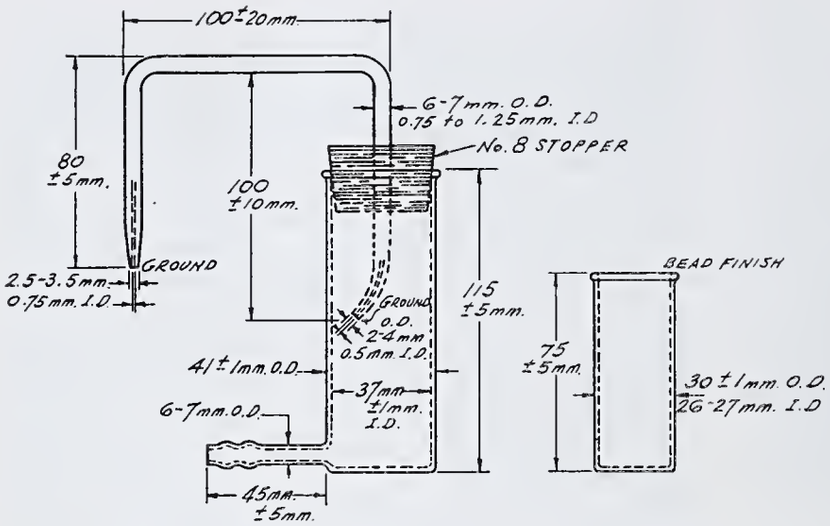

FIGURE 6. SIPHON, RECEIVER, AND INNER CONTAINER FOR BARIUM SULFATE FILTRATION

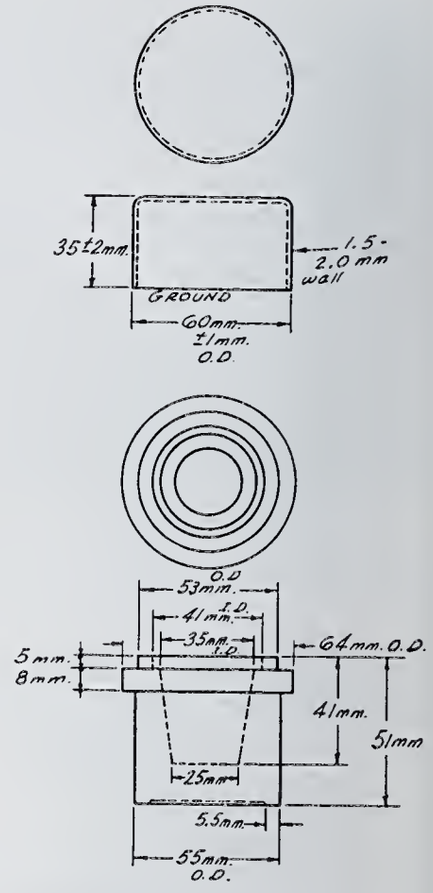

FIGURE 7. GLASS DOME AND METAL CRUCIBLE DESICCATOR

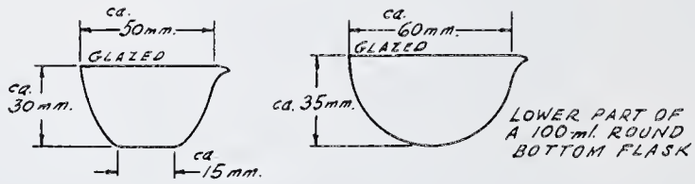

FIGURE 8. MICRO EVAPORATING DISH FOR SULFUR BOMB DETERMINATION

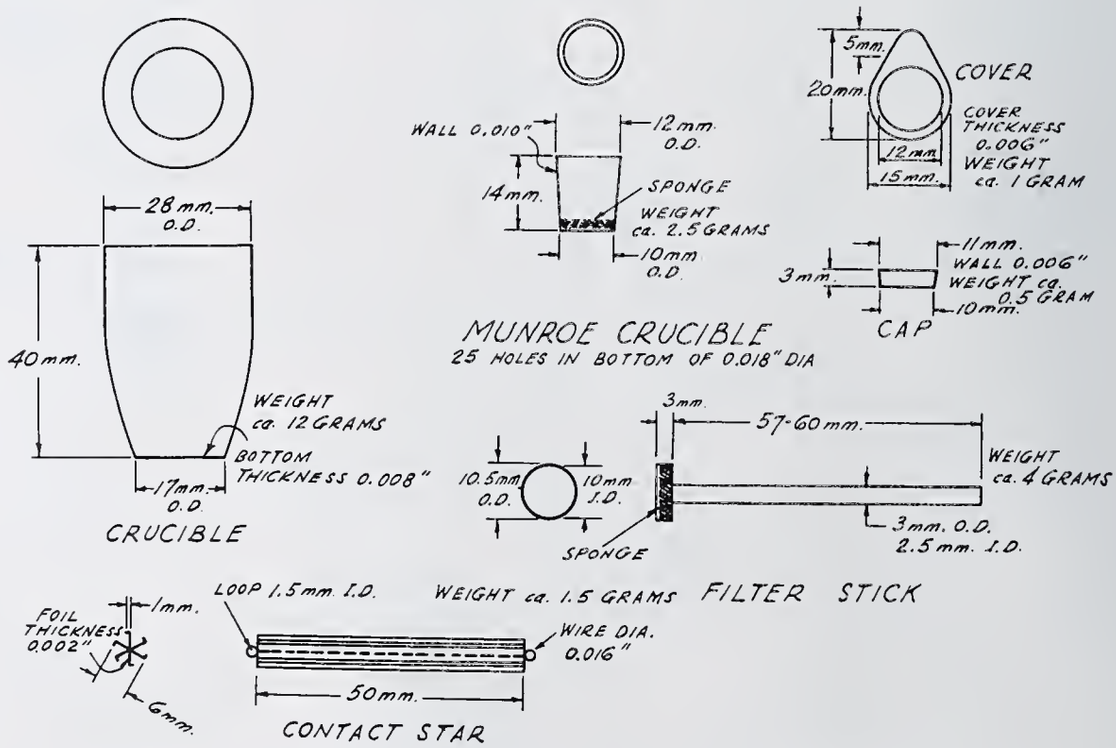

FIGURE 9. PLATINUM MICROWARE FOR HALOGEN AND SULFUR DETERMINATIONS

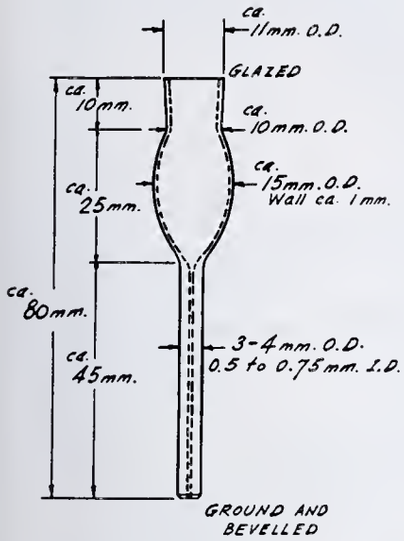

FIGURE 10. AIR FILTER

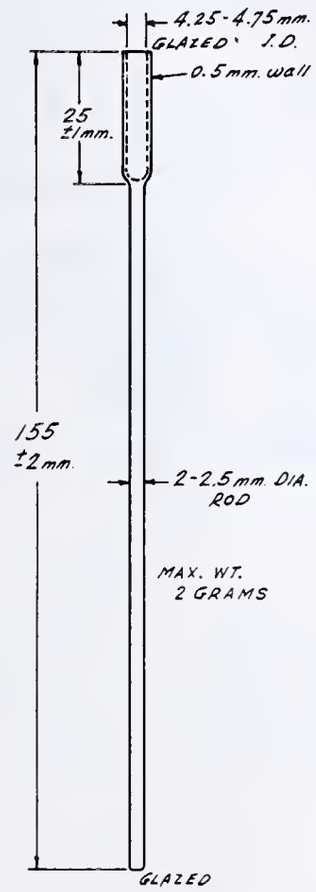

FIGURE 11. WEIGHING TUBE

Tube for Carius like above, except 200 mm. over-all

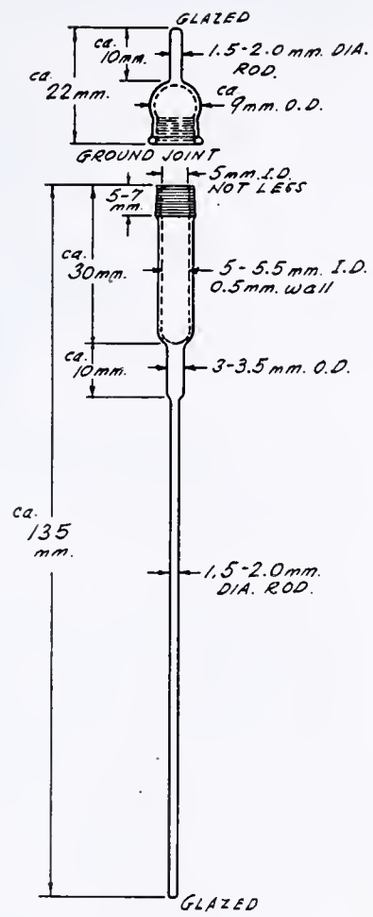

FIGURE 12. WEIGHING TUBE WITH CAP

Part IV. Halogen

For the determination of halogens, the committee considered the following specifications necessary:

- Air filter (Figure 10)
- Weighing tube (Figure 11)
- Weighing tube with cap (Figure 12)
- Snipe feather (Figure 13)
- Combustion tube (Figure 14)
- Filter tube (Figure 15)
- Filtration assembly (Figure 16)
- Graduated wash bottle (Figure 17)
- Sintered-glass funnel (Figure 18)
- Large-size glass test tube for metal microbomb (Figure 19)

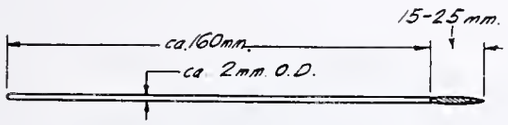

FIGURE 13. SNIPE FEATHER

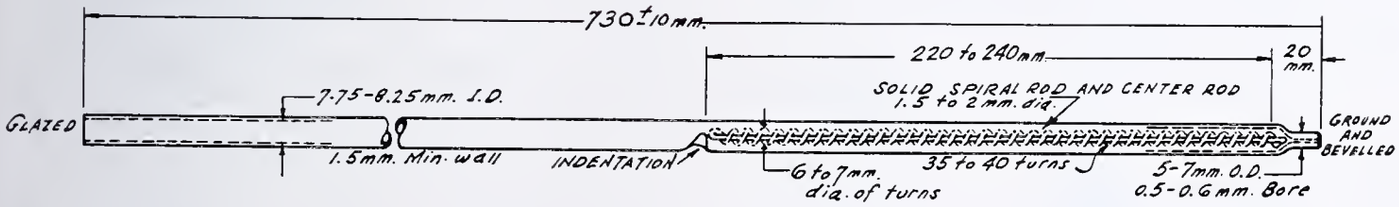

FIGURE 14. COMBUSTION TUBE

Acknowledgment

It is not possible to give credit to the originator of every piece of apparatus which has been considered, nor to acknowledge all the help which the committee has received in the formulation of the report. However, the committee wishes to express its appreciation of the help and financial aid received from the Division of Analytical and Micro Chemistry and others who have given personal suggestions.

Literature Cited

(1) Royer, G. L., *et al.*, IND. ENG. CHEM., ANAL. ED., 13, 574 (1941).

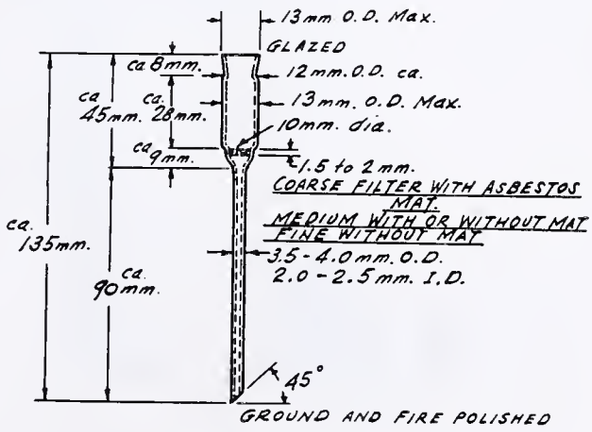

FIGURE 15. FILTER TUBE

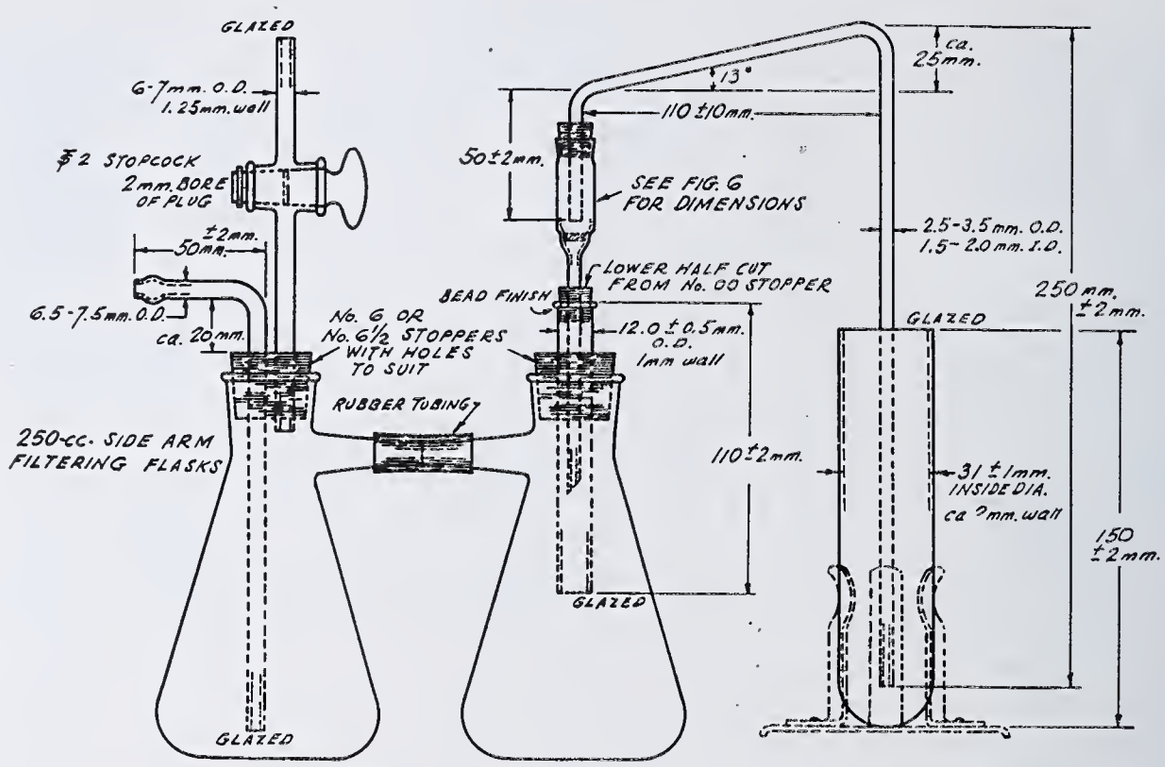

FIGURE 16. FILTRATION ASSEMBLY

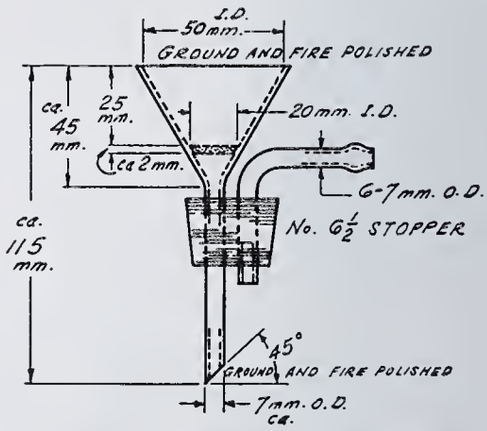

FIGURE 18. SINTERED-GLASS FUNNEL

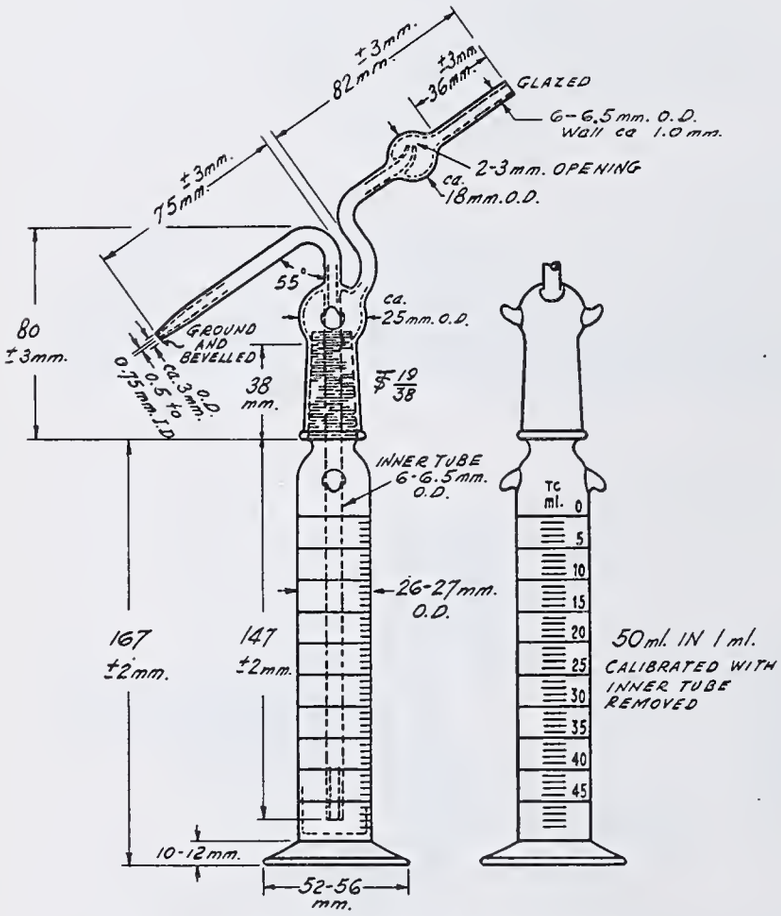

FIGURE 17. GRADUATED WASH BOTTLE

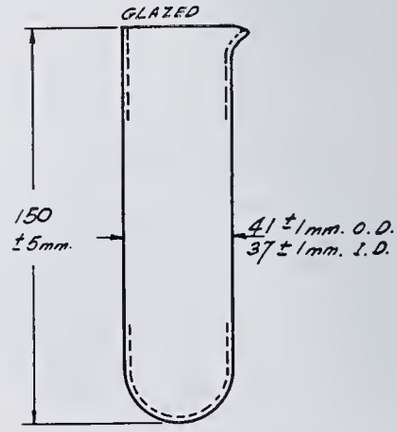

FIGURE 19. LARGE-SIZE GLASS TEST TUBE FOR METAL MICROBOMB

INDUSTRIAL AND ENGINEERING CHEMISTRY

ANALYTICAL EDITION

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The Viscosity of Pitches

W. F. FAIR, JR., Mellon Institute, AND E. W. VOLKMANN, Koppers Company, Pittsburgh, Penna.

The viscosities of the less fluid bituminous materials are usually determined at elevated temperatures, under which conditions the consistencies may be convenient for estimation with empirical or capillary-type viscometers. To determine the viscosity of such materials at lower temperatures a simplified falling coaxial cylinder viscometer was devised, following the recommendations of Traxler and co-workers. Results obtained for a group of pitches indicate that these pitches exhibit viscous flow at 25.0° C., and have better temperature susceptibilities below their softening points than at higher temperatures. Viscosities calculated from the Saal viscosity-penetration relation do not agree with the results obtained with the falling cylinder viscometer.

Similar studies on a special pitch distillate demonstrate that this material changes upon standing with progressive increase in apparent viscosity and probable development of yield values.

THE absolute viscosities of tars and pitches may be readily determined at different temperatures by means of capillary rise viscometers as shown by Volkmann, Rhodes, and Work (*6*), and the change in viscosity with temperature thereby studied. However, the practical use of this method is restricted to temperature ranges in which the bituminous material under investigation is fluid enough to exhibit a measurable rise in a reasonable period of time, corresponding roughly to temperatures above the ring and ball softening point of the material. As a linear relation between log log viscosity and log absolute temperature has been found to hold for tars and pitches in this temperature interval by Rhodes, Volkmann, and Barker (*3*), confirming the earlier work of Ubbelohde and associates (*5*), it has been thought that viscosities at lower temperatures might be estimated by extrapolation of curves based on results obtained at the higher temperatures. The slope of this linear curve furnishes a convenient numerical reference for temperature susceptibility.

Because the normal use of many tars and pitches depends upon their properties at much lower temperatures than the ones at which the capillary rise method can be applied, it was determined to try to investigate the viscosities of these materials at lower temperatures by some other method, if possible, to determine whether or not the extrapolated viscosity values were of the correct order of magnitude.

After some time spent on studying the various methods which might give reliable results, it was decided to employ a simplified falling coaxial cylinder viscometer, such as has been described by Traxler and Schweyer (*4*).

Two instruments were made, one of brass, *B*, and one of aluminum, *A;* each set of cylinders rested upon a base equipped with detachable rods, upon which additional weights might be suspended, so that a wide range of weights could be used, and the material under examination thus subjected to widely differing stresses. The apparatus had the following dimensions: the height, *L*, of both inner and outer cylinders, was 2.54 cm., the inner radius, *R*, of the outer cylinder was 1.905 cm., and the radius, *r*, of the inner cylinder was 1.270 cm., as recommended by Traxler and Schweyer. The low-weight aluminum cylinder was used when the effects of relatively lower shearing stresses were being studied.

To make a determination, the space between the two concentric cylinders was filled with the molten pitch, which was allowed to cool to room temperature, then the excess material was trimmed off with a hot spatula, and the instrument was suspended in a constant-temperature bath. The viscometer assembly was placed upon a stand which supported the outside cylinder only, thus allowing the inner cylinder to fall at a slow rate, depending upon the nature of the material in the annular space, and the total weight of the inner cylinder, stem, and base, plus any suspended additional weights. The drop of this inner cylinder was observed by following a mark near the top of the upright stem attached through the inner cylinder to the base, by means of a micrometer microscope.

In Figure 1 are shown the filled *B* set of cylinders, three weights, two rods, the *A* set of cylinders, and the stem. In Figure 2 the viscometer is illustrated fully assembled but supported by a wire attached to the top of the stem to prevent any movement while the sample is coming to temperature. In Figure 3 the viscometer is presented as it appears while a determination is being made, with the inner cylinder and attached base and stem free to fall.

After observing the distance, *h*, in centimeters, through

Table I. Softening Points

Sample	Softening Point (Ring and Ball) ° C.
1	53.4
2	55.1
3	52.2
20	56.8
4	56.0
5	57.0

which the inner cylinder dropped in t seconds, under the influence of total weight, W, in grams, the viscosity is calculated by substituting in the following equation:

$$V = \left(\log_e \frac{R}{r}\right)\left(\frac{g}{2\pi L}\right)\left(\frac{W \times t}{h}\right)$$

For the specified dimensions of the instrument described above, this expression reduces to

$$V = (0.4055)\ (61.466)\left(\frac{W \times t}{h}\right) = 25.0\left(\frac{W \times t}{h}\right)$$

From this formula the apparent viscosity for any given weight may be calculated. If the material being examined exhibits truly viscous flow, the viscosity will be constant, independent of the weight used; but if the material is nonviscous, the apparent viscosity will increase with decreasing weights. For this reason it is of value to know the shearing stress and the rate of shear, for a graph showing these functions indicates the apparent viscosity and the yield value. If the material exhibits viscous flow, the curve will be a straight line directed toward the origin.

These values may be calculated according to the following equations:

$$\text{Shearing stress, } F = \frac{-Wg}{2\pi L}\left(\frac{1}{R} - \frac{1}{r}\right) = 16.1 \times W$$

$$\text{Average rate of shear, } \frac{dv}{dr} = \frac{h}{t}\ (R - r) = (0.635)\left(\frac{h}{t}\right)$$

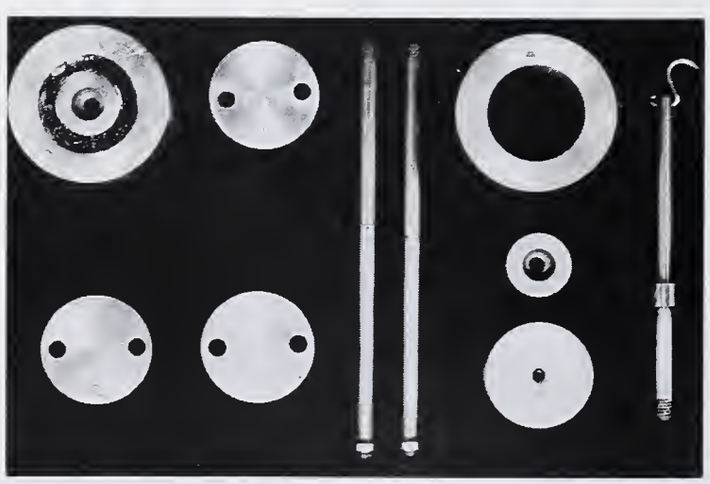


Figure 1. Cylinders, Weights, Rods, and Stem

Five different coal-tar pitches were obtained for measurement of viscosity in this apparatus and a sixth pitch, No. 20, was prepared by distilling heavy water-gas tar in the laboratory. Their softening points (ring and ball) are given in Table I.

In Table II are set forth the experimental observations and calculated results for the viscosity, shearing stress, and the rate of shear, as determined at 25° C. for these pitches. Inspection of this tabulation shows that reproducible results have been obtained, and that these pitches have constant

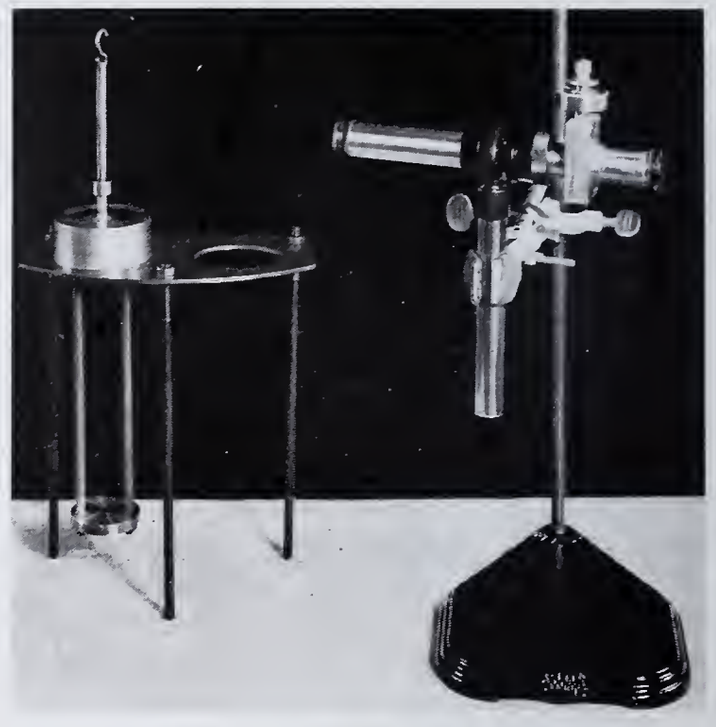


Figure 2. Assembled Viscometer

viscosity at 25° C. (within an experimental error of about 5 per cent) over a wide range of applied stresses. In Figure 4, the respective shearing stresses, F, are plotted against the corresponding rates of shear $\left(\frac{dv}{dr}\right)$; inspection of these curves clearly indicates that these pitches exhibit truly viscous flow at 25° C.

The linear curves obtained by plotting stress against rate of shear in all cases may be extrapolated to the origin, thus emphasizing the purely viscous nature of these materials.

To obtain a rough evaluation of the temperature susceptibility of these pitches at low temperatures it was decided to

Table II. Viscosity, Shearing Stress, and Rate of Shear

Sample	W Grams	Drop Mm.	Time Sec.	Viscosity Poises	F	$dv/dr \times 10^5$
5	87.0	1.32	3600	5.94×10^7	1400	2.34
	169.3	1.77	2519	6.02	2730	4.56
	216.8	1.80	1990	6.03	3500	5.84
	300.3	2.52	2033	5.89	4830	8.19
	435.1	1.91	1051	5.99	7000	11.7
Av.				5.95×10^7		
20	87.0	1.83	2498	2.96×10^7	1400	4.73
	169.3	2.47	1739	2.98	2730	9.18
	216.8	2.76	1392	2.73	3500	12.9
	300.3	3.21	1202	2.82	4830	17.1
	435.1	5.03	1318	2.84	7000	24.7
	442.4	4.83	1335	3.05	7100	23.6
Av.				2.89×10^7		
4	87.0	1.64	3635	4.82×10^7	1400	2.90
	169.3	2.49	2842	4.83	2730	5.66
	216.8	3.06	2622	4.65	3500	7.55
	300.3	3.95	2565	4.84	4830	10.0
	435.1	3.06	1319	4.69	7000	14.9
Av.				4.77×10^7		
2	87.0	3.76	3643	2.11×10^7	1400	6.64
	170.5	4.69	2269	2.13	2740	12.9
	217.5	3.98	1466	2.01	3510	17.5
	300.6	5.34	1431	2.01	4830	24.0
	435.3	5.91	1104	2.07	7000	33.8
Av.				2.07×10^7		
1	87.0	3.72	2751	1.61×10^7	1400	8.70
	170.5	4.36	1656	1.63	2740	16.8
	217.5	4.15	1188	1.57	3510	22.3
	300.6	5.48	1158	1.59	4830	30.4
	435.3	6.39	909	1.55	7000	45.1
Av.				1.59×10^7		
3	87.0	1.19	1553	2.84×10^7	1400	4.93
	217.5	2.60	1284	2.69	3510	13.1
Av.				2.77×10^7		

Table III. Temperature Susceptibility

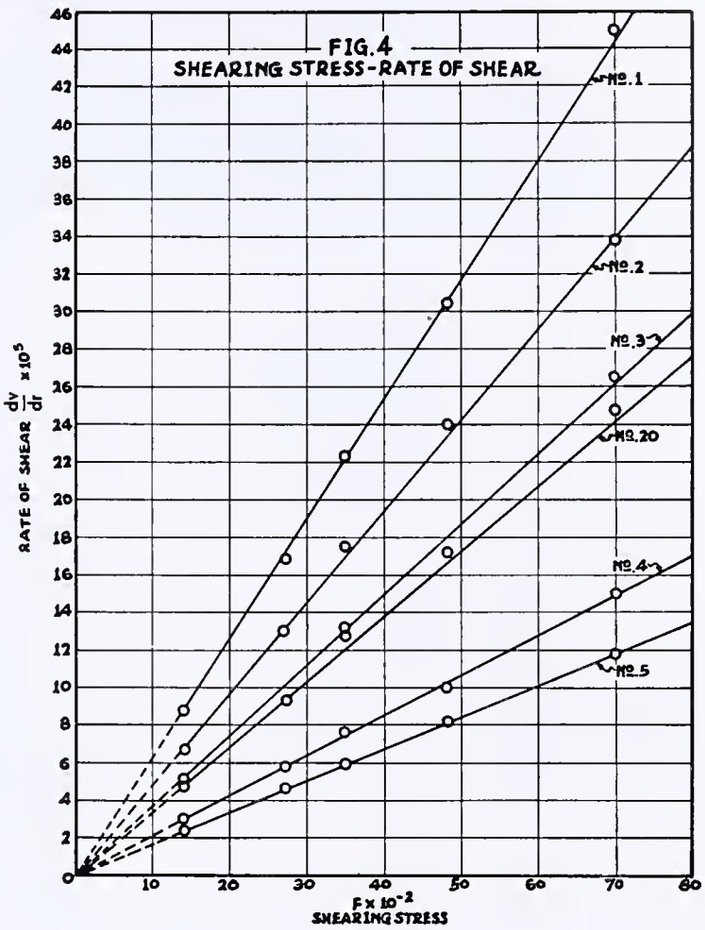
Sample	Softening Point ° C.	Viscosity at Softening Point *Centistokes*	Viscosity at 25° C. *Centistokes*	*S*
5	57.0	1×10^6	4.68×10^9	1.04
20	56.8	1×10^6	2.33×10^9	0.98
3	52.2	1×10^6	2.25×10^9	1.12
4	56.0	1×10^6	4.00×10^9	1.05
2	55.1	1×10^6	1.67×10^9	0.99
1	53.4	1×10^6	1.30×10^9	1.02

convert the above viscosities to kinematic viscosity at 25° C. and use the approximate relation of a viscosity of 1×10^6 centistokes at the temperature of the ring and ball softening point. The values for the temperature susceptibility thus obtained are given in Table III. These values are the slopes of the straight lines obtained by plotting log log viscosity against log absolute temperature in each case.

Figure 3. Viscometer

With the exception of pitches 3 and 20, these values are somewhat lower than has previously been generally found for similar tars, where the calculation of the temperature susceptibility was based upon viscosity determinations made at higher temperatures. Assuming the softening point relation to be sufficiently accurate for these considerations, it is obvious that either the viscosity as above measured is incorrect, or the temperature susceptibility of these pitches at low temperatures is better than has been generally believed.

In continuing this investigation it was decided to determine the viscosity coefficients for some of these pitches at different temperatures by the more reliable falling cylinder and capillary rise methods. Typical results, converted to centistokes, are summarized in Table IV. From these findings it appears by indirect comparison that the falling cylinder viscometer gives values comparable to results obtained by the small capillary rise instrument. Some time after these determinations had been completed an open-end rotating cylinder viscometer as devised by Ford and Arabian (*1*) was available.



Results with this instrument agreed well with the coaxial viscometer referred to. The temperature susceptibility of these pitches at low temperatures thus appears to be better (lower) than has been previously suspected. If log log viscosity is plotted against log absolute temperature over the intervals discussed, it appears (Figures 5 and 6) that for two typical pitches the temperature susceptibility below the softening point is lower than it is above that temperature. Included for comparison (Figure 7) is a curve based on Pochettino's results roughly converted to centistokes (taken from *2*), which also seems to indicate a break in the curve near the softening point region, but in the opposite direction than was found for the two pitches discussed above; in other words, this pitch showed a poorer susceptibility at low temperatures than at high temperatures, which is just the reverse of the results found for the pitches here described. It is believed that the results described truly represent changes in susceptibility occurring in the general region of the softening point and are not due to instrumental differences, since the results of Table IV indicate that viscosity measurements

Table IV. Viscosity

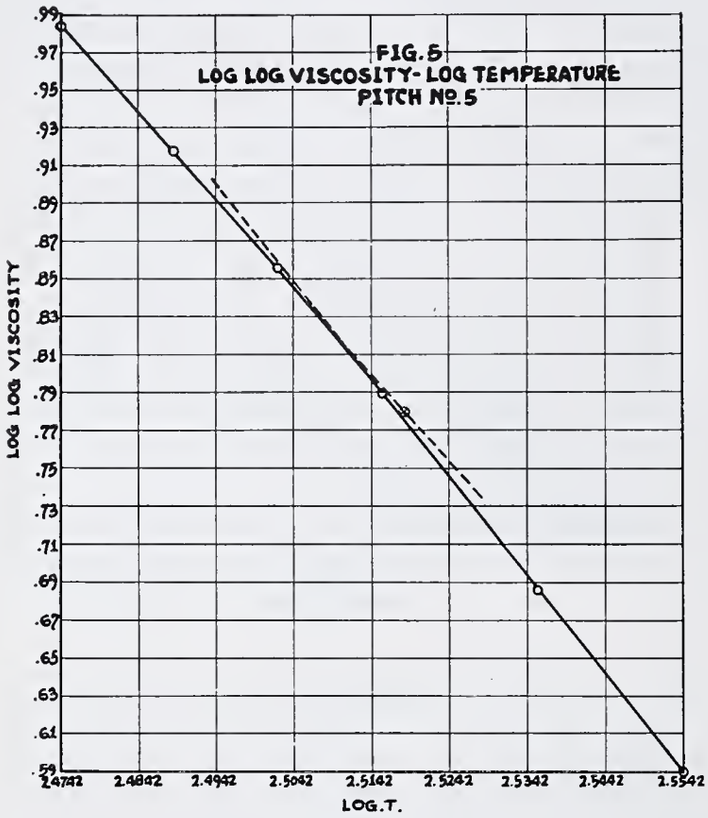
Sample	Temperature ° C.	Method	Viscosity *Centistokes*	Temperature ° C.	Method	Viscosity *Centistokes*
5	25	Cylinder	4.68×10^9	35	Cylinder	2.0×10^6
	45	Cylinder	1.55×10^7	45.1	Large capillary	1.46×10^7
	55	Small capillary	1.43×10^6	55	Large capillary	1.51×10^6
	70	Small capillary	6.84×10^4	85	Small capillary	7.81×10^3
20	25	Cylinder	2.33×10^9	35	Cylinder	1.50×10^8
	45	Cylinder	1.40×10^7	35	Large capillary	1.63×10^8
	70	Small capillary	1.23×10^5	85	Small capillary	1.55×10^4

made by the capillary and falling cylinder viscometers are in agreement.

Penetration measurements were made for these pitches and viscosity results were then calculated by the Saal relation. The results were much lower than were reported above, but the ratio of the viscosity determined by the falling cylinder to that calculated by the Saal formula seemed to decrease with increasing penetrations, as shown in Table V.

Table V. Viscosity

Sample	Penetration	Viscosity by Cylinder, 25° C. *Poises*	Ratio of Viscosities
1	27.3	1.59×10^7	1.78
2	25.2	2.07	2.00
3	22.9	2.77	2.25
20	22.7	2.89	2.28
4	18.4	4.77	2.47
5	16.1	2.95	2.44

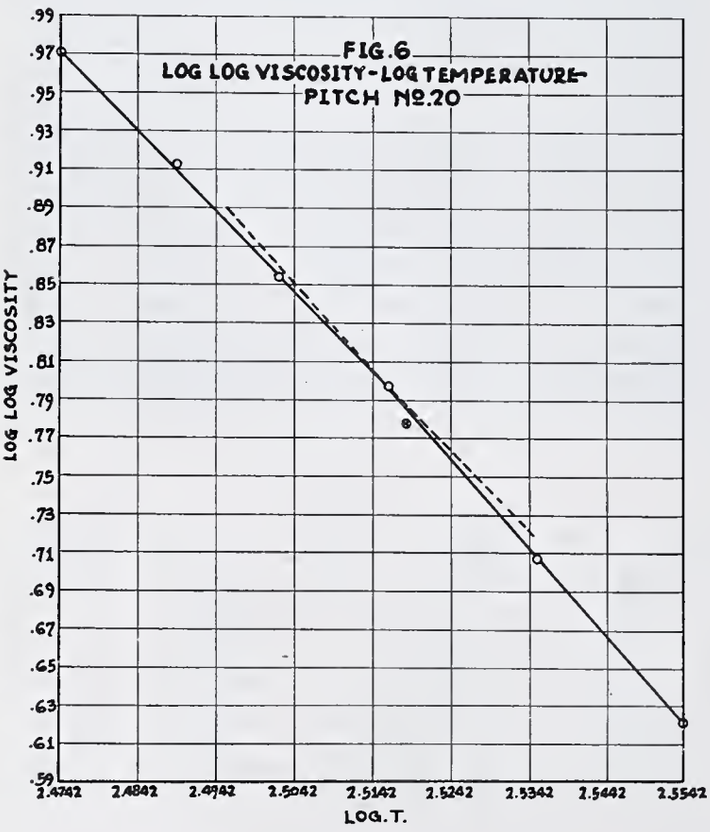


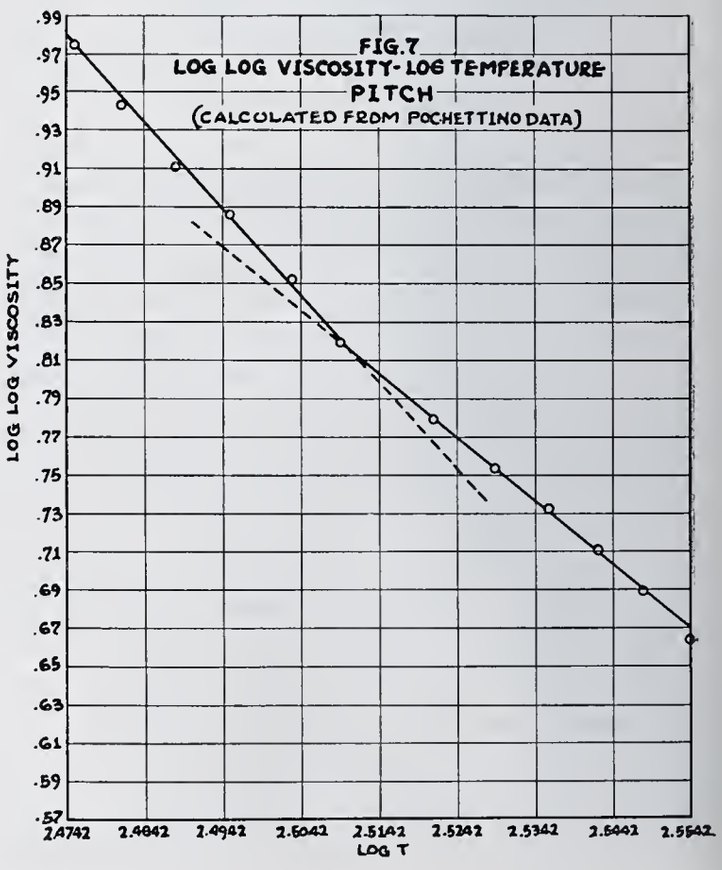
To learn whether or not the ratio of these viscosities might approach unity for softer pitches, as seems to be indicated, several pitches of lower softening points were prepared and investigated. The results obtained will be given in a subsequent paper.

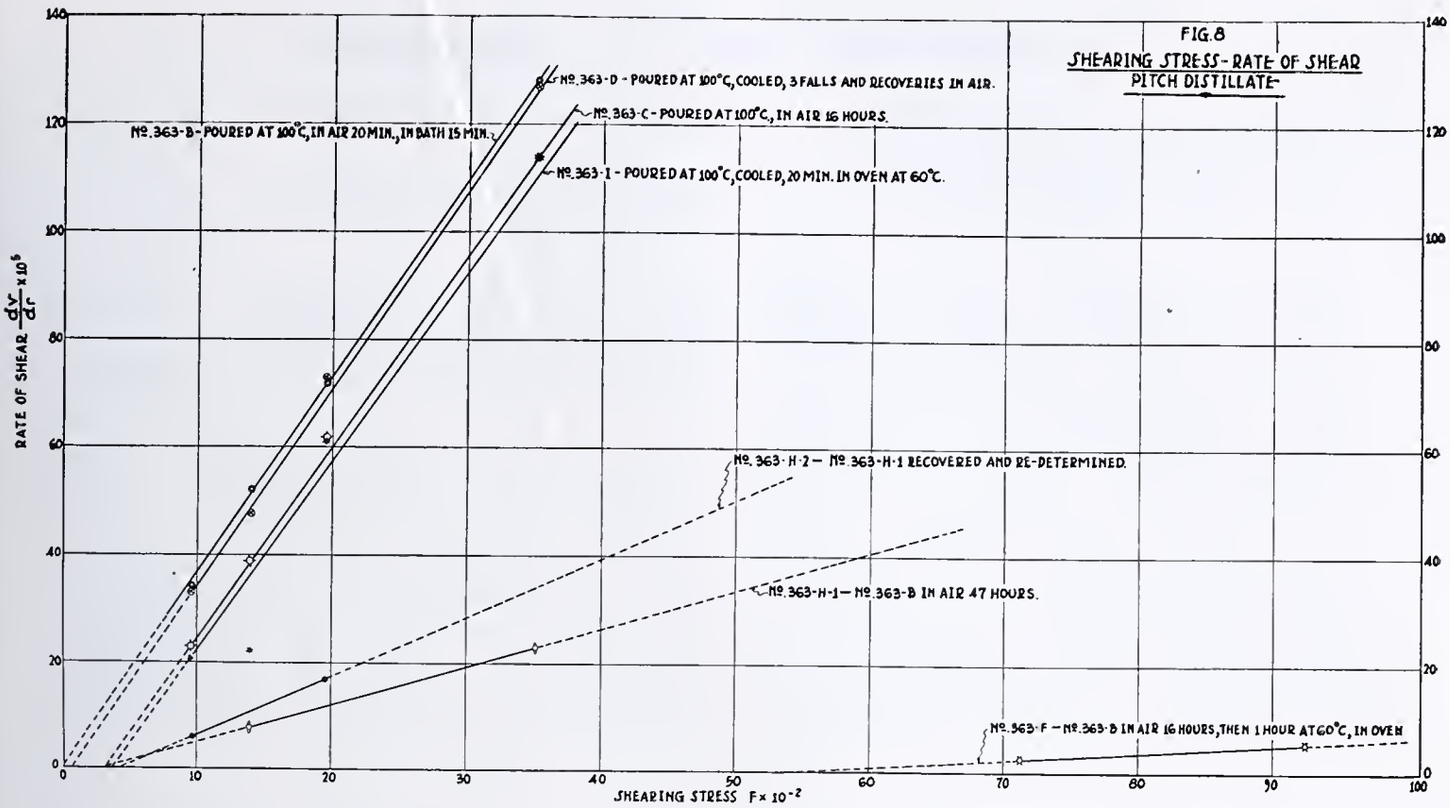
As these results agreed well with earlier reports concerning the viscous flow of pitches, it was decided to investigate, as a possible interesting contrast, the flow properties of a high-boiling pitch distillate, which is in the state of a soft paste at room temperature. Previously empirical investigations had indicated the probability of anomalous flow for this material. Viscosity determinations were therefore made at 25° C., using the falling coaxial cylinder viscometer described previously.

At first the results for pitch distillate, with a softening point (ring and ball) of approximately 43.0° C., were rather scattered, but later, when standard test procedures were arbitrarily adopted, and strictly adhered to, better agreement was obtained. A summary of the preparations, test procedures, and the experimental data is given in Table VI, and the stress-rate of shear curves is presented in Figure 8. Comments on these flow properties have been included with each set of determinations.

From these results it is obvious that the flow properties of pitch distillate will depend upon its previous treatment, and







upon the stresses to which it is subjected, as well as upon intermediate fluctuations in temperature, in all of which factors this distillate differs from residual straight-distilled tars and pitches.

Conclusions

In consequence of this investigation it is felt that the falling cylinder viscometer will prove to be a valuable tool for the determination and comparison of the rheological properties of tar products. Some typical pitches exhibit viscous flow and have better temperature susceptibilities below their softening points than has been previously thought to be the case from considerations based upon extrapolations from viscosity measurements at higher temperatures.

These tentative conclusions may be modified after future research. It is believed that continued experimentation along these lines will add materially to our present knowledge of the flow properties of tars and pitches.

Table VI. Summary of Data

Sample	W Grams	Drop Mm.	Time Sec.	Viscosity Poises	F	dv/dr × 10⁵
363-B						
(dehydrated pitch distillate, poured at 100° C., in air 20 minutes, in bath 15 minutes)	59.9	3.95	739	0.280×10^7	963	34.3
	87.0	4.39	545	0.271	1400	51.9
	121.9	4.74	423	0.272	1960	72.0
	217.5	4.52	228	0.275	3510	128.0
Av.				0.276×10^7		
363-C						
(363-B, poured at 100° C., in air 16 hours, in bath 15 minutes)	217.5	4.10	229.3	0.304×10^7	3510	114.0
	87.0	4.02	670	0.364	1400	38.6
	59.9	3.64	1030	0.424	963	22.7
	121.9	4.57	491	0.318	1960	61.7
Increased apparent viscosity, nonviscous flow, yield value about 350 dynes per sq. cm.						
363-D						
(363-B, poured at 100° C., cooled, 3 falls and recoveries in air, in bath 15 minutes)	217.5	4.58	230.2	0.273×10^7	3510	127.0
	87.0	4.66	632	0.294	1400	47.6
	121.9	4.86	431	0.269	1960	73.0
	59.9	4.27	834	0.292	963	33.0
Av.				0.282×10^7		
Slightly increased apparent viscosity, small yield value of about 100 dynes per sq. cm.						
363-F						
(363-B, in air 16 hours, then 1 hour at 60° C. in oven)	217.5	0.0	3000	(high)	3510	0
	443.4	0.62	1591	2.84×10^8	7120	2.51
	573.5	1.37	1553	1.63	9240	5.65
Greatly increased apparent viscosity, nonviscous flow, yield value of about 6000 dynes						
363-H-1						
(363-B in air 47 hours)	217.5	3.32	933	1.53×10^7	3510	22.9
	87.0	1.56	1302	1.82	1400	7.70
363-H-2						
(363-H-1 recovered, and redetermined)	121.9	3.24	1222	1.14	1960	17.2
	59.9	1.76	1866	1.59	963	6.05
Increased apparent viscosity with time, decreased by "working", yield value unchanged, remaining about 350 dynes per sq. cm. (compare with 363-C)						
363-I						
(363-B, poured at 100° C., cooled, 20 minutes in oven at 60° C., then determined)	87.0	1.77	507	0.625×10^7	1400	22.4
	217.5	4.29	242	0.308	3510	114.0
	59.9	2.79	846	0.456	963	21.1
	121.9	5.08	532	0.319	1960	61.5

Acknowledgment

The authors wish to express their appreciation to H. R. Beck and E. J. Maloney for assistance in laboratory determinations and in the preparation of the graphs.

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Viscosity of Coal-Tar Residues

Comparison of Viscosity Measurements and A. S. T. M. Penetration Tests

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The industrial chemist who examines the asphaltic and coal-tar residues commonly used for protective coatings, roofing, and waterproofing purposes determines the apparent viscosities of these materials by the A.S.T.M. penetration test. Many attempts have been made to correlate the results of this test with absolute viscosity measurements. The formula established by Saal has been accepted by many investigators as a reliable relationship as long as the materials under test do not show anomalous flow properties. A theoretical analysis of the empirical formula confirms the validity of its general form. The results of a number of viscosity determinations and the corresponding penetration values of various pitches exhibiting viscous flow are presented and correlated on double logarithmic coordinate paper. The Saal formula seems reasonably reliable above penetration values of about 60 decimillimeters. Below this value considerable deviations are apparent. This is not surprising, since the standard penetration needle is not a simple cylindrical stem but consists of a cylindrical shank with a truncated cone 54 decimillimeters long.

THE penetrometer is the instrument commonly used in commercial laboratories for evaluating the flow characteristics of materials of high consistency. The equipment and procedure are specified by the American Society for Testing Materials (*1*). The test with this instrument has the advantage that the equipment needed is simple and relatively inexpensive, and requires little skill in its operation. The disadvantage of the penetration test is its empirical nature. Results are expressed in terms of decimillimeters which have no direct relation to accepted rheological units, thus making it impossible to correlate penetration measurements with other consistency tests.

In 1933 Saal and Koens (*5*) published the results of an investigation concerning the rheological properties of some asphaltic materials. On the basis of tests conducted on two steam-distilled asphalts they came to the conclusion that in the case of "nonplastic asphaltic bitumens" the relationship between penetration measurements and absolute viscosity can be expressed by

$$\eta = \frac{5.9 \times 10^9}{(\text{penetration})^{1.93}}$$

where η is viscosity in poises and penetration is expressed in decimillimeters. This formula, known as the Saal formula, has been used by many technologists. Saal (*4*) indicated that it can be partly derived from theoretical considerations. Rhodes and Volkmann (*3*) confirmed this contention, proceeding from different assumptions.

Traxler and co-workers (*9*) inquired into the validity of the Saal formula by a carefully conducted study of the rheological properties of a series of steam-distilled asphalts selected as most closely approaching materials of normal, or Newtonian, flow characteristics. They found that in general a straight-line relationship resulted if the logarithm of viscosity was plotted *vs.* the logarithm of penetration. Each asphalt, however, gave a separate line differing from the line representing the Saal formula and from that of other asphalts. Furthermore, a closer inspection of the line resulting from connecting the experimental points for any given asphalt showed that it was actually a sinuous curve and not a straight line. In a discussion of Traxler's results Saal and Labout (*6*) insist that the application of the Saal formula is permissible only for materials of a purely viscous character and intimate that Traxler's asphalts do not fulfill this requirement. It is not surprising that in reply Traxler (*8*) claims that "this stringent limitation of the applicability of the relationship makes it worthless from an industrial standpoint. . . . ."

It is probably correct to state that most commercial asphalts exhibit some elasticity, thixotropy, or anomalous flow properties. In contradistinction straight-distilled coal-tar pitches and related materials, consisting mainly of condensed aromatic ring structures, seem to retain their normal Newtonian flow characteristics over a wider range of consistencies even upon aging. It seemed to the authors that these products should be especially suited to check the validity of the Saal formula; hence an investigation was undertaken to determine whether in the case of coal-tar pitches a definite relationship existed between penetration data and absolute viscosity measurements and, if this should be the case, to find out whether the relationship could be expressed adequately by the Saal formula.

A total of 23 samples was selected. Five of these (group A) were commercial coal-tar pitches prepared by batch-distillation of coke-oven tar in direct-fired horizontal stills. Each sample came from a different crude tar. The next four samples (group B) were coal-tar pitches prepared in the laboratory by distillation of four different crude coke-oven tars. A further group (C) of nine samples was obtained in the laboratory from one crude coke-oven tar by a single distillation, during which pitch-residue samples of increasing consistencies were withdrawn. As some of the heavy water-gas tars in their physical and chemical characteristics resemble coke-oven tars, five samples of pitches derived from such water-gas tars were also tested (group D). They were prepared in the laboratory by distillation of different crudes. Finally, as a matter of curiosity, a sample of pitch of higher softening point was also included. This pitch had a penetration (100 grams, 5 seconds, 25° C.) of 1 decimillimeter, a value below the accepted lower range limit for the instrument.

The choice of samples for the purpose at hand seemed appropriate, particularly because Traxler and Coombs (*7*) have indicated that the presence and magnitude of anomalous flow properties of asphalts depend upon the source, the type and degree of processing, and the temperatures and rates of shear at which the measurements of flow are made. The samples represent a wide variety in source and in the type and degree of processing. As the rate of shear changes with the depth of penetration and because the consistencies of the samples selected cover the whole practical range of the penetrometer, the factor of rate of shear is also varied. Furthermore, a few of the pitches (group A) used in this

investigation have been shown (*2*) to exhibit normal flow characteristics at 25° C. under different stresses.

There remains the factor of temperature. Inasmuch as the investigation has been conducted primarily to determine whether penetration test results as obtained in commercial testing laboratories can be converted into absolute viscosity units, and because the penetration test is most commonly conducted at 25° C., it was decided not to vary the temperature and to determine penetration values and absolute viscosities at 25° C.

The penetration measurements have been made in accordance with the test procedure specified by the A. S. T. M. (*1*). At least three tests were run on each material and the average was reported.

Table I. Viscosity

Sample No.	Sample Group	Penetration (100 Grams; 5 Seconds, 25° C.) *Decimillimeters*	Observed Viscosity *Poises*	Calculated Viscosity (Saal Formula) *Poises*	Deviation from Observed Value %
7	B	195	1.99×10^5	1.95×10^5	− 2.0
12	C	186	2.02×10^5	2.14×10^5	+ 5.9
11	C	185	2.12×10^5	2.16×10^5	− 1.9
22	D	156	2.48×10^5	3.00×10^5	+21.0
8	B	154	2.97×10^5	3.08×10^5	+ 3.7
23	D	138	3.74×10^5	3.80×10^5	+ 1.7
24	D	73.8	1.41×10^6	1.27×10^6	− 9.9
13	C	69.2	1.42×10^6	1.44×10^6	+ 1.4
25	D	57.5	2.42×10^6	2.06×10^6	−14.8
9	B	53.1	2.51×10^6	2.40×10^6	− 4.4
14	C	36.0	7.56×10^6	5.02×10^6	−33.6
1	A	27.3	1.59×10^7	8.67×10^6	−45.4
2	A	25.2	2.07×10^7	1.01×10^7	−51.2
10	B	23.4	2.51×10^7	1.17×10^7	−53.3
3	A	22.9	2.77×10^7	1.22×10^7	−55.9
20	D	22.7	2.89×10^7	1.24×10^7	−57.0
15	C	19.9	3.49×10^7	1.60×10^7	−54.1
4	A	18.4	4.77×10^7	1.86×10^7	−61.0
5	A	16.1	5.95×10^7	2.40×10^7	−59.8
16	C	14.1	7.11×10^7	3.11×10^7	−56.2
17	C	9.3	1.99×10^8	6.93×10^7	−65.2
18	C	5.2	4.28×10^8	2.13×10^8	−50.2
19	C	3.1	7.99×10^8	5.78×10^8	−27.6
31	..	1.0	3.2×10^9	3.29×10^9	+ 2.8

The viscosities have been determined with the falling coaxial cylinder instrument described in a previous paper (*2*).

The results are presented in Table I and Figure 1. In Table I the materials are listed in the sequence of their ascending consistencies. In the fifth column is shown the viscosity calculated by the Saal formula from the penetration as determined for each sample, and in the last column the deviation of the calculated from the observed viscosity is recorded.

An inspection of the values given in the last column shows that for penetration readings of more than 50 decimillimeters the data derived from the Saal formula are in fair agreement with the observed viscosity data. The greatest deviation is 21 per cent and the average deviation is 6.7 per cent. The agreement is excellent if the residues of group D are excluded—i. e., if only residues of coal-tar origin are considered. In that case the maximum deviation is only 5.9 per cent and the average deviation 3.2 per cent.

But for penetration readings of less than 50 decimillimeters there is considerable divergence between the observed viscosity data and the values derived from the Saal formula. The experimental data with one exception all seem to fall on a smooth curve (see Figure 1), indicating that the divergence is caused not by the appearance of anomalous flow properties but by factors not considered in the Saal formula. In all probability the physical shape of the penetrometer needle is responsible for the failure of the formula at readings below 50 decimillimeters. The needle consists of a cylindrical shank with a truncated cone at its lower extremity. If, in the course of a penetration measurement, the needle drops to a depth greater than the length of the truncated cone (5.4 mm.), the resistance to further immersion probably is determined principally by the cylindrical shaft. Under those conditions a simple relationship between viscosity and penetration, such as the Saal formula, may well hold,

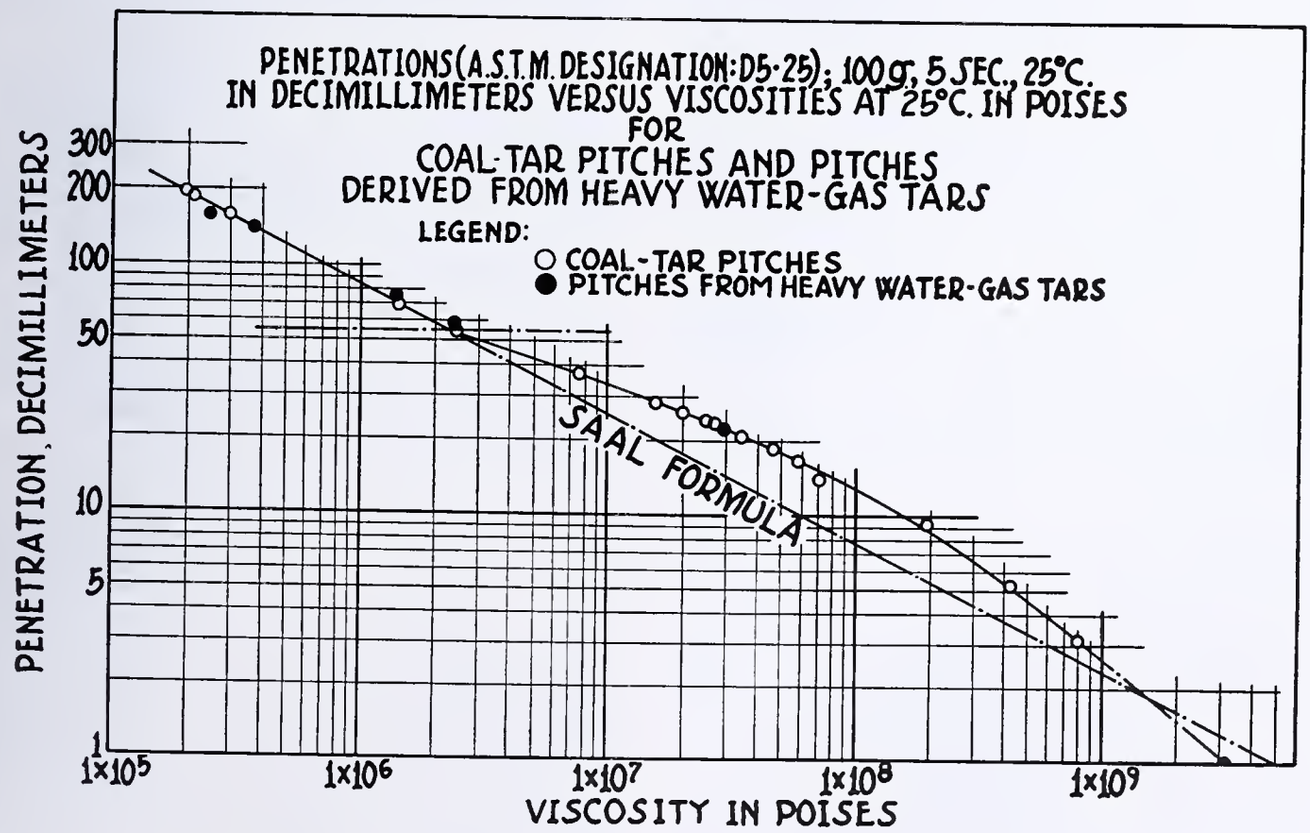

Figure 1. Viscosity Determinations with Falling Coaxial Cylinder Instrument

at least for materials of the type tested. If, however, only the conical portion of the needle is partially immersed, conditions are much more complex and the same relationship cannot be expected to apply.

Conclusion

It can be concluded that, in the case of coal-tar pitches and, to a less extent, of pitches from heavy water-gas tars, there is a definite relationship between standard A. S. T. M. penetration measurements (100 grams, 5 seconds 25° C.) and absolute viscosity. This relationship is well expressed by the Saal formula only for penetration readings above 55 decimillimeters. Below this value the formula is not applicable.

Acknowledgment

The authors wish to express their appreciation to H. R. Beck, G. W. Brown, and E. J. Maloney for assistance in laboratory determinations and in the preparation of the graphs.

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Determination of Benzene

Detection and Estimation of Benzene in the Presence of Toluene, Xylene, and Other Substances

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THE known chemical methods for the determination of benzene do not appear to be applicable in the presence of its homologs, especially toluene and xylene. The sulfuric acid–formaldehyde (*4*), nickel cyanide (*7*), dracorubin (*2*), levulose (*10, 11*), butanone extraction (*13*), *m*-dinitrobenzene reduction (*5, 12, 14, 15*), and Stepanow colorimetric methods (*1*) are not specific for benzene. Physical methods for detection of benzene, such as fractionation or ultraviolet absorption (*3*), likewise cannot be used to estimate the concentration of benzene when it is mixed with unknown concentrations of other substances including its homologs. The method described below was developed in order to satisfy the need (*6, 8, 9*) of being able to ascertain the presence and concentration of benzene in mixtures of various materials as it is commonly encountered in industry.

Briefly, the method consists of nitration, extraction with ether, development of color with butanone and alkali in alcoholic solution, elimination of the color due to interfering substances by the addition of acetic acid, and comparison of the residual color.

Benzene when nitrated in the manner described below is almost completely converted to *m*-dinitrobenzene. This compound when treated with butanone and sodium hydroxide in alcoholic solution produces a crimson color. Toluene, when similarly treated, is partly converted to the dinitro compound and forms a blue color which quickly turns violet; xylene forms a relatively permanent deep blue color. After addition of acetic acid, the color due to benzene remains; the colors due to toluene and xylene disappear rapidly. No color is generated with the aliphatic hydrocarbons and their derivatives such as methanol, ethanol, ethyl acetate, isopropyl alcohol, butanol, butyl acetate, acetone, and similar substances found in such commercial products as paints, lacquers, solvents, and thinners.

Reagents and Apparatus

Nitrating mixture, equal parts by volume of fuming nitric acid, specific gravity 1.49 to 1.50, and concentrated sulfuric acid, specific gravity 1.84.

Sodium hydroxide aqueous, 1 volume of 40 per cent sodium hydroxide diluted to 4 volumes with distilled water.

Sodium hydroxide alcoholic made by diluting 1 volume of 40 per cent sodium hydroxide to 10 volumes with 95 per cent alcohol and filtering just prior to use.

Acetic acid, made by diluting 1 volume of glacial acetic acid to 10 volumes with 95 per cent alcohol.

Butanone (methyl ethyl ketone), practical grade.

Set of test tubes of about 50-ml. capacity. These tubes should be of the same diameter (2 cm.) and equal transparency. A photoelectric colorimeter may be used if available.

Pipets graduated to 0.01 ml.

Procedure

A 0.50-ml. portion of the solution to be analyzed is measured into a 50-ml. Erlenmeyer flask. The flask is placed in a freezing mixture which consists of chopped ice and salt at a temperature of 0° to −5° C. Nitrating mixture from a buret is added to the solution at the rate of 2 drops per second while the flask is rotated. After 10 ml. have been added, the flask and contents are removed from the ice mixture and 35 ml. of distilled water are added.

The resulting mixture is cooled to room temperature and the nitrated hydrocarbons are extracted with ether, by shaking the mixture with 25 ml. of ether in a 125-ml. separatory funnel and repeating the extraction of the aqueous layer three times with 10 ml. of ether each. The ether layers are transferred into a 100-ml. volumetric flask. The combined ether solutions are poured into the separatory funnel and washed with 10 ml. of aqueous sodium hydroxide reagent, then consecutively with 2 portions of 10 ml. of

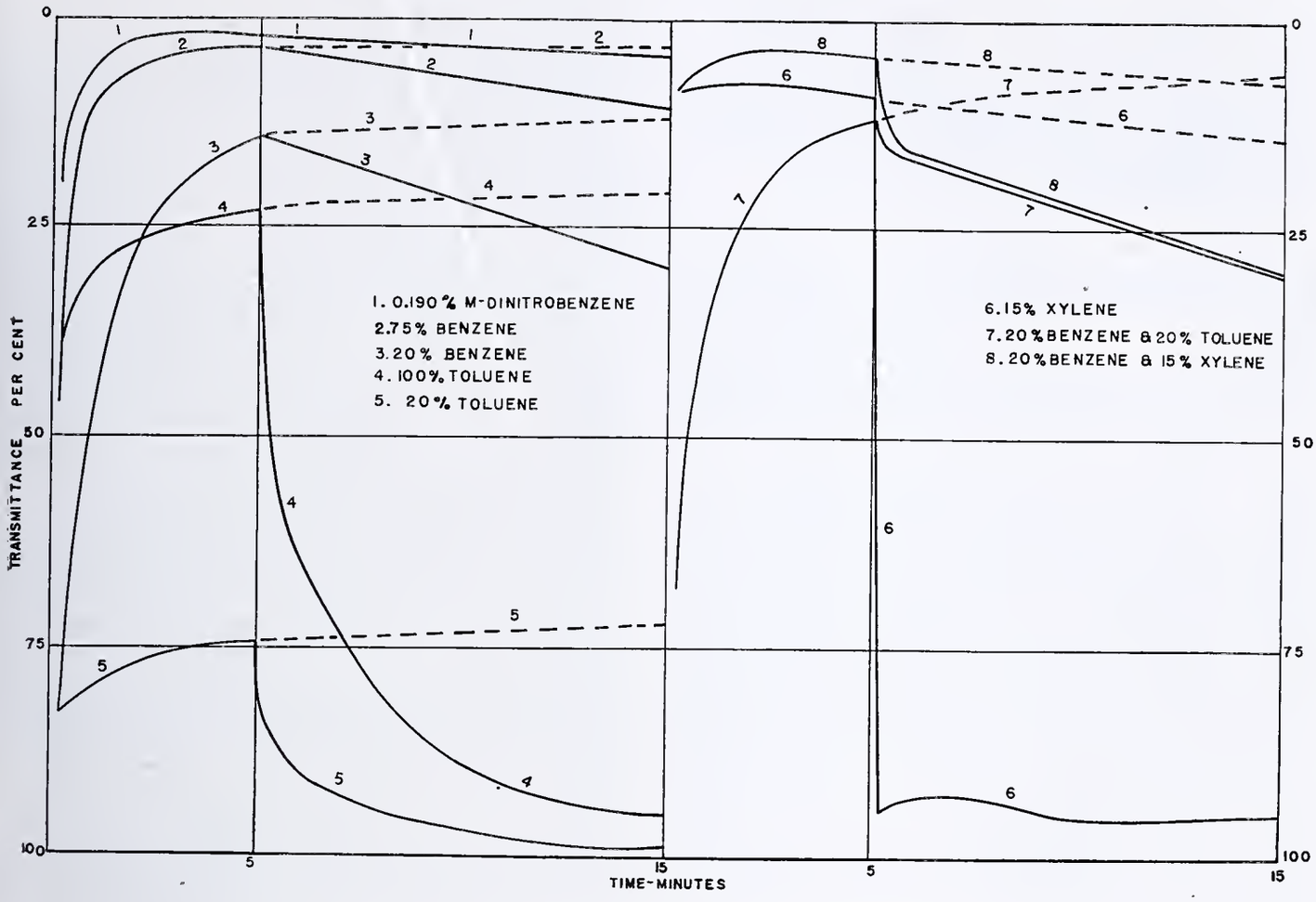

FIGURE 1. RATE OF COLOR DEVELOPMENT AND DETERIORATION

distilled water. The washed ether solution is then returned to the 100-ml. flask and 95 per cent ethyl alcohol is added to make a total volume of 100 ml. A 10-ml. aliquot of this solution is pipetted into a 50-ml. volumetric flask and 95 per cent alcohol added to the 50-ml. mark.

A 10-ml. portion of the last solution is now measured into one of a set of similar glass tubes. One or more 10-ml. samples of known concentrations of nitrated benzene made as above are also measured into other tubes of the set. To each are added 1.0 ml. of butanone and 0.50 ml. of alcoholic sodium hydroxide reagent. The tubes are agitated and after 5 minutes the colors are observed. Portions of 0.50 ml. of acetic acid reagent are now added to each tube. The tubes are again agitated and after 10 minutes the color is compared with that of the standard most closely approaching it in intensity.

The comparison may be made as follows: A measured quantity of alcohol is added from a buret or pipet to the solution of greater intensity until both tubes match exactly. If the test solution has a yellow tinge, as is found when high concentrations of xylene are encountered, a dilute solution of sodium or potassium dichromate placed in back of the tube containing the known concentration is of considerable aid in matching the two solutions. The quantity of alcohol added must be taken into consideration. If after 2.5 ml. of alcohol have been added to the test solution, it matches the standard containing the equivalent of 10 per cent benzene, the concentration of benzene in the sample $= \frac{12 + 2.5}{12} \times 10 = 12.1$ per cent.

As an alternative to the above, which may be called the dilution method of comparison, a set of standards of varying concentrations may be used. This is known as the standards method of comparison. Standards—e. g., 12.0, 12.5, 13 per cent, etc.—may be obtained from 15 or 20 per cent benzene by diluting the alcoholic solution of nitrated benzene with requisite volumes of alcohol. Butanone, sodium hydroxide, and acetic acid reagents must be added to these standards at the same time and under the same conditions as in the case of the solution whose benzene concentration is to be determined.

The intensity of color may be compared with the aid of an appropriate colorimeter such as the Evelyn photoelectric colorimeter with filter No. 620. Readings are taken on the galvanometer scale 5 minutes after the sodium hydroxide reagent has been added (A), and again 10 minutes after the acetic acid reagent has been added (B). The concentration of benzene is then read directly on a curve previously plotted as concentration of benzene *vs.* light transmittance or readings on the galvanometer scale. If the difference in the two readings—i. e., $B - A$—is not very great, no correction is necessary. If, however, the difference is considerable, an empirically obtained correction is applied. If the reading A is 0, the color should be developed on a more dilute solution. This may be obtained by taking a smaller volume of alcohol-ether solution—e. g., 5 ml. instead of 10 ml.—and adding alcohol to a final volume of 10 ml. in the test tube before adding the alkali and acid reagents. This dilution must, of course, be taken into account in the computation.

The reference curve which is used with the photoelectric colorimeter is plotted from the readings obtained on solutions of varying known concentrations of benzene. These solutions are prepared by diluting C. P. benzene with purified petroleum naphtha and treating them in the manner described above and under similar conditions.

The analysis need not take more than 30 minutes. The nitration takes about 2 minutes. The dilution, cooling, extraction, and washing usually consume about 10 minutes, since the stratification of the ether and aqueous layers is prompt. Little more than 15 minutes is required for the production of color and the procurement of the necessary data when the photoelectric colorimeter is used. In the absence of a colorimeter, the additional time consumed in the use of standards is short. Once prepared, the standard solutions keep well when guarded from solvent evaporation by the use of glass-stoppered flasks. They can be used for numerous analyses, since the withdrawal of only 10 ml. is required for a determination.

Characteristics of Benzene, Toluene, and Xylene

The curves in Figure 1, as well as in Figures 2 and 3, represent data obtained by measuring with the aid of a photoelectric colorimeter (Evelyn with filter No. 620) the intensity of color developed by the procedure described above. All readings were taken at temperatures 24.5° ± 0.4° C.

Curves 2, 3, 4, 5, and 7, when representing solutions in alkaline media (broken lines, 5 to 15 minutes after sodium hydroxide reagent, but no acid, had been added) do not reach their maxima within 15 minutes. Curves 6 and 8 reach their maxima shortly before 5 minutes. However, the rate of color development which is represented by the ordinates of light transmittance is considerably decreased at the end of the 5-minute period. The rate of color deterioration in the acid media is sufficiently low 10 minutes after the acid has been added, or at a total of 15 minutes after color development was started, to require no additional delay for taking final readings.

Curve 1 in Figure 1 represents results obtained with 10 ml. of a solution of 0.190 gram of Eastman Kodak *m*-dinitrobenzene in 100 ml. of solution of 95 per cent U. S. P. ethyl alcohol. Curve 2 was derived from a solution prepared by nitrating 0.50 ml. of C. P. benzene, then adding 2.5 ml. of alcohol to 7.5 ml. of its thousandfold diluted alcoholic solution and treating it with butanone, alkali, and acid, as prescribed. This solution contains an equivalent of 75 per cent of benzene. Curve 3 was drawn from data obtained on a solution composed of 20 volumes of C. P. benzene diluted to 100 volumes with purified petroleum naphtha. Curve 5 was obtained by similar treatment of toluene. Purified petroleum naphtha, in all cases, was prepared by shaking commercial petroleum naphtha and benzine with 100 per cent sulfuric acid to remove aromatic hydrocarbons. The oil was then washed with distilled water, dried with anhydrous potassium carbonate, and distilled. Curve 6 was plotted with data from 15 per cent of xylene in purified petroleum naphtha. Curves 7 and 8 were furnished, respectively, by solutions containing 20 per cent benzene plus 20 per cent toluene, and 20 per cent benzene plus 15 per cent xylene in purified petroleum naphtha. In this paper per cent specified is by volume, unless otherwise stated.

The rate of color development of benzene, when treated by the above procedure, is rapid at first, then gradually diminishes. After addition of the acid reagent the color remains the same, though decreased somewhat in intensity (curves 2 and 3, Figure 1). The color formed with the solution of *m*-dinitrobenzene which corresponds in concentration to that given by 100 per cent benzene, if completely nitrated to *m*-dinitrobenzene, shows the same characteristics (curve 1, Figure 1). In contrast, the color of the toluene complex develops slowly, does not reach the intensity produced by equal concentrations of benzene, and fades rapidly after adding the acid reagent (curves 4 and 5). Xylene, at a 15 per cent concentration, almost instantaneously exhibits a color nearly as intense as 75 per cent benzene. However, 15 seconds after adding the acid reagent, its blue color completely disappeared (curve 6).

The slight interference resulting from the presence of toluene and xylene is illustrated by curves 7 and 8 in Figure 1. In alkaline media, both curves differ considerably from curve 3, although they all contain 20 per cent benzene. After the acid reagent is added, all curves rapidly assume the form given by 20 per cent benzene despite the fact that one solution contains 20 per cent of toluene and another 15 per cent of xylene in addition to 20 per cent of benzene.

Varying concentrations of benzene, toluene, and xylene produce different colors and intensities of color when alkali reagent is added to the alcoholic solutions of butanone and the nitrated compounds. The contrast between benzene on one hand and toluene and xylene on the other becomes much more pronounced after the acetic acid reagent is added. This is illustrated in Figure 2. The broken lines here represent results 5 minutes after the sodium hydroxide reagent had been added. The solid lines represent results obtained 10 minutes after the acetic acid reagent had been added or a total of 15 minutes after color development had begun. Curves 1 and 3 demonstrate the resemblance of the xylene to the benzene reaction when in alkaline media. This resemblance is practically nullified 10 minutes after the acetic acid has been added. Benzene largely retains its properties—i. e., of an exponential curve $y = ae^{-kx}$ (curve 4, Figure 2)—whereas the curves for toluene and xylene are transformed into straight lines located at the lower section of the graph.

The concentration of either toluene or xylene can also be determined by this method, provided only one of these and no benzene or other chromogenic compound is present. Better results, however, in this case are obtained when the color comparison is made 5 minutes after the sodium hydroxide reagent has been added, and the broken line curves in Figure 2 are used.

Mixtures of these compounds can thus also be qualitatively ascertained. A solution of benzene and toluene gives a purplish violet color, whereas a solution of benzene and xylene gives an opaque violet color. After addition of the

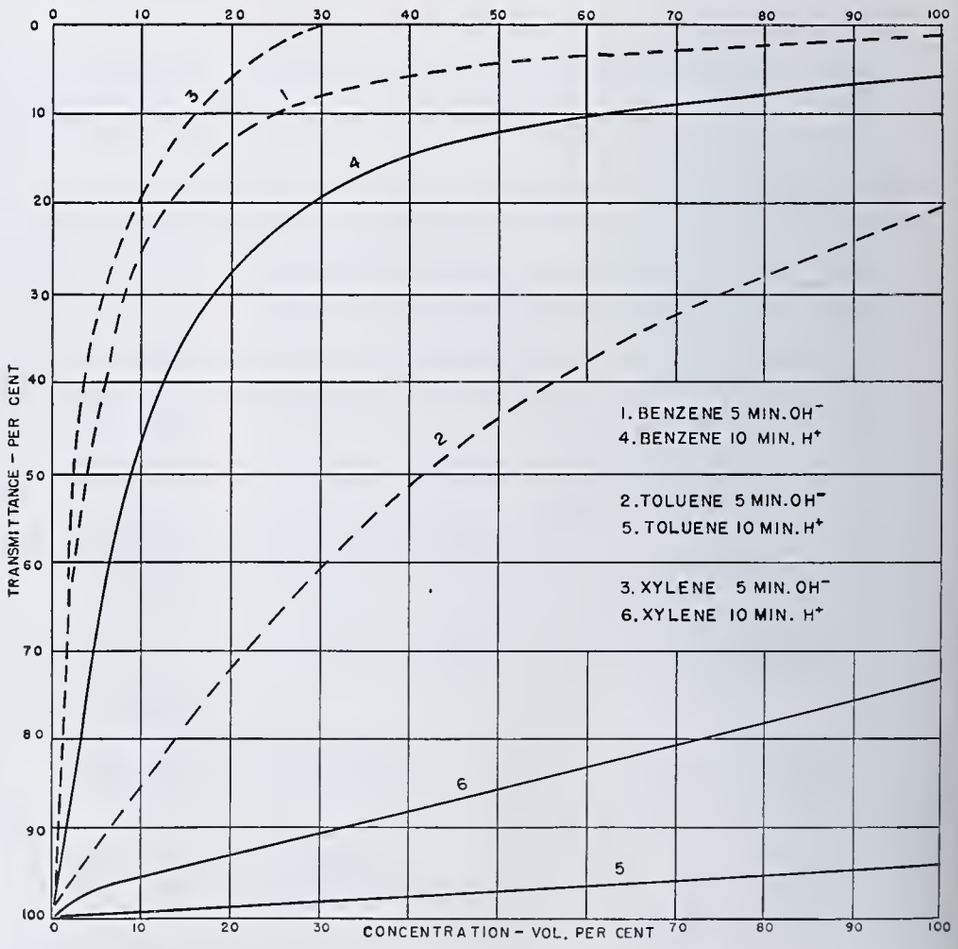

FIGURE 2. RELATIONSHIP OF CONCENTRATION TO INTENSITY OF COLOR

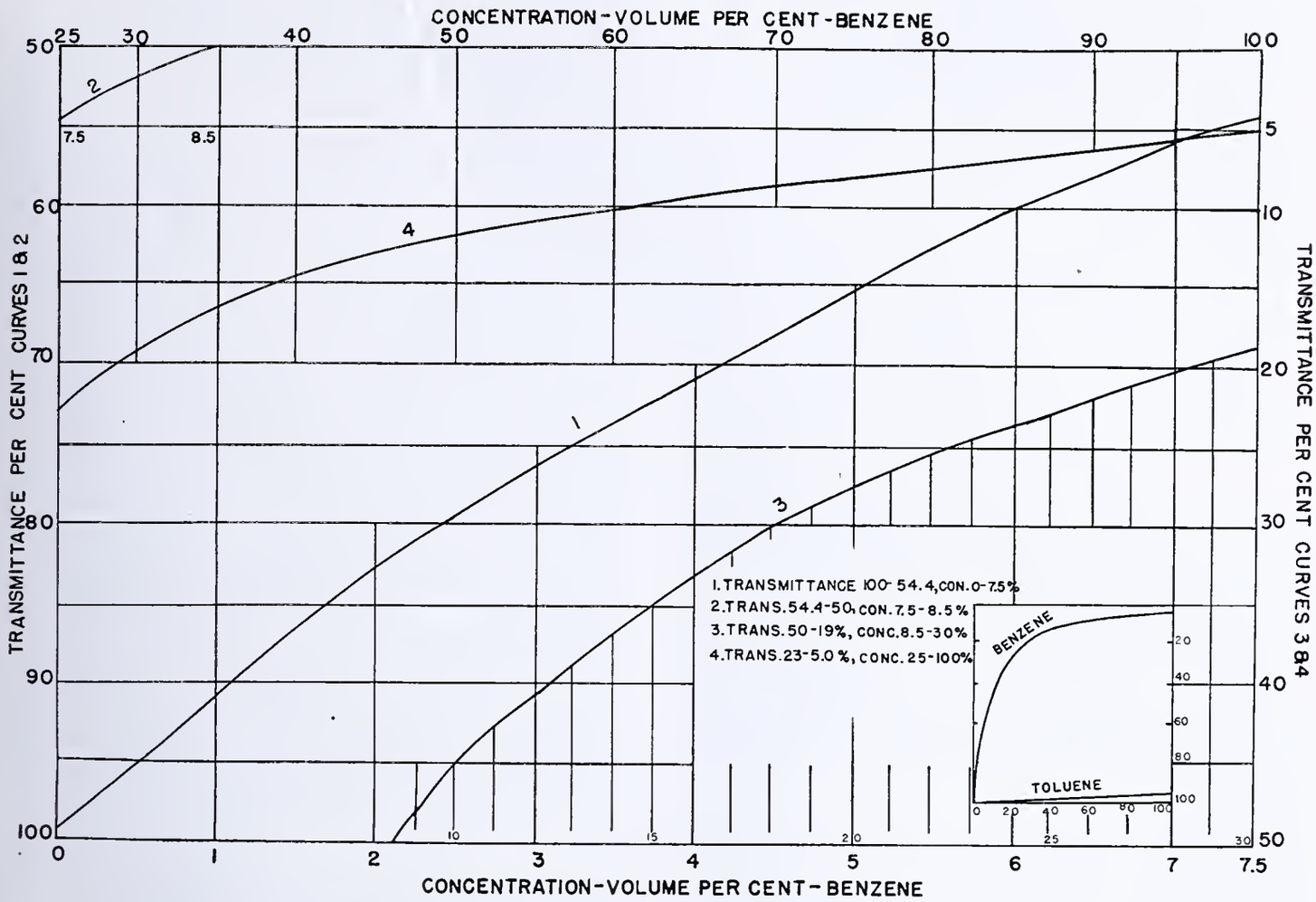

FIGURE 3. REFERENCE CURVE FOR PHOTOELECTRIC COLORIMETER

acid reagent the crimson color gradually appears in the case of benzene and toluene, whereas it almost immediately emerges in the case of benzene and xylene solutions. On observation with the unaided eye, the intensity of color indicates the concentration of chromogen. When the photoelectric colorimeter is used, wide differences between the 5- and 15-minute readings indicate high concentrations of either toluene or xylene or both. The character and position of a curve plotted as light transmittance *vs.* time will indicate the concentration of either toluene or xylene or both.

Curve 4 of Figure 2 is the reference curve mentioned under Procedure above. Figure 3 shows this curve broken up in four sections plotted on three different scales for the abscissa. This was necessitated by the fact that the constants a and k of the curve $y = ae^{-kx}$ are applicable for limited sections of the curve. The formula $y = mx + b$ or a straight-line function, actually describes some sections of the curve best. Furthermore, the use of the curves in Figure 3 facilitates readings of benzene concentrations with an error no greater than the maximum error inherent in the manipulations. The complete curve is shown at the lower right corner of the graph. The complete curve obtained from similar treatment of C. P. toluene was inserted here for comparison. In view of the fact that the benzene curve rises very sharply—i. e., the difference in color intensity for a given increment of benzene is greater—from 0.01 to 30 per cent of benzene this range was plotted in the form of three different curves. The section for 25 to 100 per cent of benzene was plotted on a fourth curve to a scale coarser than that of curves 3, 2, and 1, but finer than the similar curve in Figure 2.

Checking of Method

In order to ascertain the accuracy of the method, solutions containing different concentrations of benzene and other substances were prepared. The results obtained are shown in Tables I to IV.

TABLE I. DETERMINATION OF BENZENE BY DILUTION METHOD OF COMPARISON

Sample No.	Composition of Samples: Benzene	Toluene	Xylene	Other	Benzene Found	Error	Error
	Volume per cent				*Vol. %*	*Vol. %*	*%*
1	75.0	25.0	..	...	77.0	2.0	2.7
2	50.0	20.0	30.0	...	50.8	0.8	1.6
3	40.0	60.0	..	...	39.8	−0.2	0.5
4	30.0	70.0	..	...	30.7	0.7	2.3
5	25.0	25.0	25.0	Petroleum naphtha 25.0	24.8	−0.2	0.8
6	20.0	..	80.0	...	19.7	−0.3	1.5
7	10.0	90.0	..	...	10.0	0.0	0.0
8	5.00	95.0	..	...	5.00	0.0	0.0
9	1.00	..	..	99.0	1.00	0.0	0.0
10	1.00	10.0	69.0	Butyl acetate 20.0	1.04	0.04	4.0
11	0.25	..	..	52.50	0.24	−0.01	4.0
				Ethyl acetate 25.85			
				Isobutyl alcohol 21.40			
12	0.20	..	..	Petroleum naphtha 99.80	0.19	−0.01	5.0

Table II. Determination of Benzene by Standards Method of Comparison

(Standards used: equivalents of 1 to 10% benzene by volume)

Sample No.	Composition of Samples: Benzene	Toluene	Xylene	Petroleum naphtha	Benzene Found	Error	Error
	Volume per cent				Vol. %	Vol. %	%
1[a]	50.0	50.0	..	..	50.0	0.0	0.0
2[a]	40.0	60.0	..	..	39.0	−1.0	2.5
3[a]	30.0	..	70.0	..	31.0	1.0	3.3
4[a]	20.0	..	80.0	..	20.0	0.0	0.0
5	10.0	90.0	..	..	10.0	0.0	0.0
6	10.0	..	..	90.0	9.9	−0.1	1.0
7	5.00	95.0	..	..	5.0	0.0	0.0
8	3.00	..	97.0	..	2.9	−0.1	3.3
9	2.00	98.0	..	..	2.0	0.0	0.0
10	1.00	99.0	..	..	1.0	0.0	0.0
11	1.00	99.0	..	..	1.05	0.05	5.0

[a] Diluted 10 times with alcohol before comparison.

The standards used for visual comparison of the test solutions in Table I were those corresponding to 0.2, 1.0, 5.0, 10.0, 20.0, and 50.0 per cent benzene. The standard used in each case was that most closely approaching in color the test solution. In the case of Nos. 1, 2, 4, 5, 9, and 11 a small quantity of alcohol gradually added to the test solution produced a color that matched the standard. Alcohol greater than this quantity reduced the intensity of color. The formula used in these cases was $B = \frac{12 + a}{12}S$, where B = volume per cent of benzene, S = benzene concentration of standard used, and a = ml. of alcohol added to match the color of the standard solution. In the case of Nos. 3, 6, and 12 the standards had to be diluted. The formula used in the latter case was $B = \frac{12}{12 + a}S$.

Table II illustrates the results obtained with test solutions compared by the alternative visual method of standards comparison. A series of standards corresponding to the range of 1 to 10 per cent benzene with unit intervals was first used. The color was then reproduced simultaneously with several standards of concentrations with increments corresponding to 0.1 per cent of benzene varying from the standard most closely matching the test solution. Whenever the concentration of the test solution appeared to be greater than 10 per cent benzene, the test solution was diluted tenfold with alcohol, the color redeveloped, and the comparison repeated.

The determinations listed in Tables III and IV were made with the aid of the graph in Figure 3. The errors found when differences in readings between the 5- and 15-minute intervals were considerable were greatly minimized by the use of empirically derived corrections. The correction applied in Table IV as well as the final results was multiplied by 10, since the test solutions were diluted tenfold. The formula used in computation for the series in Table III was benzene % = $C - (B - A - 25)\ 0.005$; the formula used for the series in Table IV was benzene % = $10[C - (B - A - 25)\ 0.005]$. C is benzene per cent corresponding to reading B on galvanometers. B and A are as given under Procedure.

Accuracy and Sensitivity

The presence of petroleum naphtha, ethyl acetate, butyl acetate, isobutyl alcohol, acetone, toluene, and xylene did not interfere with the determinations of benzene in the experiments tabulated. Reasonable accuracy, with a mean error of 1.6 per cent, was attained when the intensity of color produced was matched without the aid of a colorimeter. Greater accuracy was attained with the aid of a photoelectric colorimeter where a mean error of 0.9 per cent for a similar range of concentrations was in evidence. Concentrations less than 0.2 per cent benzene could not be determined with the naked eye, whereas concentrations down to 0.01 per cent benzene could be determined by the use of a photoelectric colorimeter. The errors involved at concentrations less than 0.2 per cent benzene, however, were considerable and amounted to 10 per cent at 0.05 per cent benzene and more at lower concentrations.

The 10-ml. aliquot of solution used for color development represents 0.01 ml. of the sample of 0.50 ml. taken for analysis and diluted to 100 × 5. Since 0.01 per cent benzene can be detected, the method appears to be sensitive to 1×10^{-6} ml. or 8.8×10^{-7} mg. of benzene. By visual comparison, in the absence of a photoelectric colorimeter or when an error above 1 per cent is not permissible, the sensitivity is reduced to 2×10^{-5} ml. or 1.8×10^{-5} mg. of benzene.

The concentration of benzene vapor in air may be determined by the above method after the vapor has been transformed from the gaseous to the liquid phase. This may be accomplished by adsorbing or dissolving the vapor in petroleum naphtha or alcohol. The concentration of benzene in the resulting solution may then be determined as prescribed. By sampling 5 liters of air, as little as 1.8×10^{-5} mg. per liter or 0.0056 part per million of benzene in air should be possible of detection. Concentrations of 0.018 mg. or more of benzene per liter of air (5.6 p. p. m.) can thus be determined with an error less than 1 per cent on a sample of 100 ml. of air.

Greater accuracy for concentrations lower than 0.2 per cent benzene or 0.018 mg. per liter of air may be obtained by decreasing the dilution. If the ether extract of nitrated solution is diluted only to 50 ml. and a 10-ml. aliquot is taken from it for the butanone reaction, 0.02 per cent, and 0.018 mg. or more per liter of air may accurately be determined on a sample of only 10 ml. of air. Concentrations down to as low as 8.8×10^{-4} mg. per liter of air or 0.27 p. p. m. may be detected on 10-ml. samples of air. The significance of this may be realized when it is perceived that numerous samples of air may thus be taken in a comparatively short time and that many small portable containers may be used.

Sources of Error

The products formed on treating benzene with nitrosulfuric acid depend not only on the volume of acid used

Table III. Determination of Benzene with Photoelectric Colorimeter

Sample No.	Composition of Samples: Benzene	Toluene	Xylene	Petroleum naphtha	Other	Benzene Found: As read on curve	Corrected	Error	Error
	Volume per cent					Vol. %		Vol. %	%
1	75.0	25.0	...	...	...	75.3	...	0.3	0.4
2	50.0	...	...	50.0	...	50.2	...	0.2	0.4
3	5.00	40.0	10.0	5.00	Butyl acetate 40.0	5.08	4.99	−0.01	0.2
4	2.00	50.0	48.0	...	...	2.29	2.01	0.01	0.5
5	1.00	99.0	...	...	...	1.12	0.99	−0.01	1.0
6	0.250	...	...	...	52.5 Ethyl acetate 25.85 Isobutyl alcohol 21.4	0.25	...	0.00	0.0
7	0.050	...	...	80.0	4.28 Butyl acetate 10.50 Ethyl acetate 5.17	0.065	0.055	0.005	10.0
8	0.010	0.06	0.03	81.9	4.00 Butyl acetate 5.00 Isobutyl alcohol 4.00 Acetone 5.00	0.030	0.020	0.01	100

Table IV. Determination of Benzene with Photoelectric Colorimeter

Dilution × 10 (10,000 total)

Sample No.	Composition of Samples: Benzene	Toluene	Xylene	Petroleum naphtha	Other	Benzene Found: As read on curve	Corrected	Error	Error
	Volume per cent					Vol. %		Vol. %	%
1	75.0	25.0	..	...	..	7.49	74.9	−0.10	0.1
2	50.0	20.0	30.0	...	..	5.16	50.1	0.10	0.2
3	25.0	25.0	25.0	25.0	..	2.67	24.9	−0.10	0.4
					Acetone				
4	20.0	..	..	...	80.0	2.03	20.3	0.30	1.5
5	20.0	40.0	20.0	20.0	..	2.19	19.8	−0.20	1.0
6	10.0	90.0	..	...	..	1.00	10.0	0.00	0.0
7	10.0	..	90.0	...	..	1.21	9.70	−0.30	3.0
8	1.00	85.0	14.0	...	..	0.305	1.05	0.05	5.0
					Butyl acetate				
9	1.00	50.0	10.0	9.00	30	0.250	1.00	0.00	0.0

and the temperature surrounding the solution but also on the rate of adding the acid. The heat formed in the reaction must be allowed sufficient time to dissipate. Too rapid addition of the nitrating acid tends to form undesirable by-products with a lower yield of *m*-dinitrobenzene.

m-Dinitrobenzene is soluble in ether to the extent of 6.7 grams per 100 ml. at 15° C. It is soluble in water to the extent of 0.047 gram per 100 ml. at 15° C. Although most of the nitrated compound enters the ether layer on extraction, some of it tends to remain in the aqueous layer. The favorable distribution of *m*-dinitrobenzene between ether and water is probably adversely affected by the mutual miscibility of the two solvents. However, even with this effect, a single extraction might be sufficient were it possible to separate the two phases completely. To ensure reasonably complete extraction, four ether extractions are made, and only small volumes of water are used to wash the ether extract.

Increase in temperature, as is often the case in chemical reactions, hastens the production of color in alkaline media and the disappearance of color in the acid media in the case of the dinitrobenzene as well as the nitrated toluene and xylene. Light also has some effect on the rate of color development and deterioration. The temperature should be within 0.4° C., and light conditions and the time elapsed should all be the same when the readings are taken with the photoelectric colorimeter as when the reference curve was prepared. When the color is compared by visual inspection, the unknown and standard are subjected to the same conditions and the effect is the same on all solutions. A series of permanent standards made from dyes or inorganic salts is not recommended because this would be applicable for only one given set of conditions.

The sensitivity of the individual observer to fine gradations of color and color intensity will influence the magnitude of error resulting from visual color comparison. This personal error, which inevitably accompanies all colorimetric determinations, may be eliminated by the use of the photoelectric colorimeter.

The photoelectric colorimetric determinations listed in Tables I and II show mean errors of 0.4 and 1.2 per cent, respectively, for concentrations above 0.2 per cent of benzene. The lower apparent accuracy in Table II is not due so much to the added manipulation of additional dilution as to the resultant decrease in benzene concentration of the test solutions. This dilution, necessitated by the fact that the solutions tested developed too intense a color in the × 1000 dilution, may perhaps be obviated by the substitution of a more appropriate filter than that used in the above experiments.

Since the galvanometer scale used can be read only to the nearest 0.25 division, the accuracy of the apparatus is limited in the presence of very high or very low concentrations of chromogen. The probability of an error of 2 per cent in 95 per cent benzene, for example, is high, even if all precautions are carefully taken. In the case of high transmittance or low concentrations of benzene, an experimental error of the magnitude of 0.005 per cent may produce an error of 10 per cent in material containing 0.05 per cent benzene. It would thus seem advisable to read the concentration of benzene at light transmittance between 20 and 80 per cent in order to avoid the upper and lower extremes of the reference curve. This may be attained by adjusting the dilution of the test solution after a preliminary determination.

Summary

A method developed for the estimation of benzene in the presence of toluene, xylene, and other substances requires little material for analysis, is rapid, and is sensitive to 8.8×10^{-7} mg. of benzene.

Concentrations varying from 0.25 to 75 per cent of benzene by volume have been determined with a mean error of 0.9 per cent.

The method may be used for the determination of small air samples.

Means for the identification of toluene, xylene, and benzene have been given.

The accuracy of the method, the sources of error, and the precautions to be taken in order to minimize the effect of the errors are discussed.

Acknowledgment

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Rapid Determination of Soluble Nutrients in Soil and Plant Extracts

By Means of a Photoelectric Colorimeter

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A KNOWLEDGE of the amounts of soil and plant nutrients soluble in weak acids, buffered salt solutions, or solutions of buffered salts and acids helps materially in explaining crop production. Because of the number of samples usually involved and the immediate need for the information, rapid tests for the soluble nutrients have been used (*1–9*, *11*, *12*). The majority of these methods depends upon a visual comparison of colors or turbidities and as such can only differentiate between certain levels of nutrients, such as low, medium, etc.

In the course of studies on the correlations of crop yields with soluble nutrients, it became necessary to define the amounts of nutrients more accurately, and to obtain quick results with large numbers of samples. Consequently, the rapid methods herein described have been adopted, and have been used satisfactorily to determine soluble nutrients in over 1000 soil samples and a similar total number of bean, pea, tomato, asparagus, and cover crop samples.

The methods employed are variations of tests already in use. They are adapted to a photoelectric colorimeter with a resulting increase in accuracy without too great a loss in speed. The nutrients in both soil and plants are extracted with Morgan's Universal extracting solution (*8*) and are determined directly in separate aliquots of the extracts. Essentially, the tests for potassium, calcium, and magnesium are variations of those employed by Morgan (*8*); the tests for nitrate nitrogen and phosphorus are those used by Carolus (*1*) and Emmert (*2*, *3*).

Apparatus

Photoelectric colorimeter with filters. A Fisher electrophotometer was used with success, and the amounts of extracts and reagents indicated are based on this machine.

Photometer tubes. Specimen vials, 80 × 25 mm., are satisfactory, if proper selection is made. Photometer readings of tubes filled with distilled water should not vary more than 0.5 unit (arithmetic scale). Tubes for nitrate nitrogen determination should be marked at 25 ml.

Water bath with 3 removable racks, each to hold 24 photometer tubes (Figures 1 and 2).

Wooden racks, each to hold 25 photometer tubes.

Automatic burets for dispensing solutions.

Drying oven.

Glass stirring rods. Pointed rods 12.5 × 0.3 cm. (5 × 0.125 inch) are satisfactory for the nitrate, phosphorus and calcium tests. Rods, 12.5 × 0.47 cm. (5 × 0.188 inch), with a flat bottom 0.94 cm. (0.375 inch) in diameter are desirable for the magnesium tests.

Pipets, 1-, 5-, and 10-ml., marked at 0.1 ml. A 10-ml. bacteriological pipet is desirable for pipetting plant extracts for calcium or phosphorus determinations.

Funnel vials, Arthur H. Thomas Co., blueprint No. 2132-1.

Wooden racks to hold 12 funnel vials.

Waring Blendor.

Reagents and Solutions

All reagents are of C. P. grade unless otherwise indicated. Wherever possible Baker's analyzed reagents were used.

EXTRACTION. Morgan's Universal extracting solution (*8*), normal sodium acetate buffered at pH 4.8 with acetic acid.

Carbon, activated, Darco grade 0–97.

DETERMINATION OF NITRATES. Phenoldisulfonic acid. Sodium hydroxide, 15 per cent. Standard nitrate, 0.2428 gram dissolved and made up to 1 liter with extracting solution = 40 p. p. m. of nitrate nitrogen.

DETERMINATION OF PHOSPHORUS. Ammonium molybdate, 2.5 per cent in 6 *N* sulfuric acid.

Aminonaphtholsulfonic acid. Fifteen grams of sodium bisulfite, anhydrous, are dissolved in 100 ml. of distilled water and 0.5 gram of pure, dry 1 amino-2 naphthol-4 sulfonic acid and 1.5 grams of anhydrous sodium sulfite, are added. The contents are shaken, made up to 500 ml., and stored in a brown bottle (*15*).

Standard phosphorus. Monosodium phosphate, monohydrate (0.0890 gram) dissolved and made up to 1 liter with extracting solution = 20 p. p. m. of phosphorus.

DETERMINATION OF POTASSIUM. To make sodium cobaltinitrite, 25 grams of cobalt nitrate and 150 grams of sodium nitrite are dissolved in 250 ml. of water containing 12.5 ml. of glacial acetic acid. The volume is diluted to 500 ml. with distilled water. The solution is allowed to stand 24 hours, then filtered, and stored in a brown bottle at 5° C.

Isopropyl alcohol, 900 ml. mixed with 100 ml. of formaldehyde (35 per cent).

Standard potassium. Potassium chloride (0.0955 gram) dissolved and diluted to 1 liter with extracting solution = 50 p. p. m. of potassium.

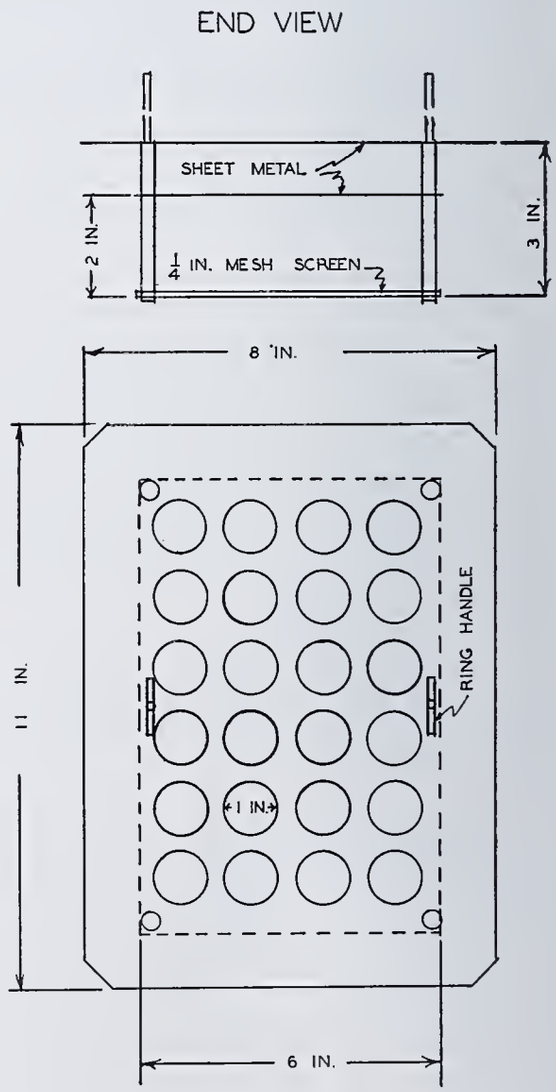

FIGURE 1. TRAY

Table I. Determination of Nutrients for Standard Curves and in Soil and Plant Extracts

Nutrient Determined	Material	Useful Range *P. p. m.*	Volume of Aliquots *Ml.*	Diluted to *Ml.*	Filter Used	Null Adjustment with Blank to
Nitrate nitrogen	Standard solution	0 to 20	0 to 2.5	5	425	100
	Soil extract	0 to 40	2.5 or 5			
	Plant extract	0 to 40	2.5 or 5			
Phosphorus	Standard solution	0 to 10	0 to 20	20	425	0 (log scale)
	Soil extract	0 to 40	5			
	Plant extract	0 to 10	20			
Potassium	Standard solution	0 to 50	0 to 10	10	650	100
	Soil extract	0 to 250	1			
	Plant extract	0 to 250	1			
Calcium	Standard solution	0 to 100	0 to 20	20	425	100
	Soil extract	0 to 600	1			
	Plant extract	0 to 30	20			
Magnesium	Standard solution	0 to 10	0 to 10	20	525	90
	Soil extract	0 to 200	1			
	Plant extract	0 to 200	1			

Determination of Calcium. Sodium oxalate, 2 per cent, prepared daily, kept in a stoppered bottle and shaken before use.

Standard calcium. Calcium acetate, dihydrate (0.4395 gram) dissolved and diluted to 1 liter with extracting solution = 100 p. p. m. of calcium.

Determination of Magnesium. Titan yellow (0.2 gram) is dissolved in 200 ml. of 50 per cent methyl alcohol solution and stored in a brown bottle. The solution will keep for about 3 months.

Sodium hydroxide, 15 per cent (same as for nitrate nitrogen).

Standard magnesium. Magnesium acetate, tetrahydrate (0.0881 gram) dissolved and diluted to 1 liter with extracting solution = 10 p. p. m. of magnesium.

Methods

Extraction of Samples. *Soil.* Twenty-five milliliters of extracting solution are added to 12.5 grams of sieved (2-mm.), air-dry soil placed in a 50-ml. Erlenmeyer flask. The contents are shaken for 1 minute and filtered on a Whatman No. 1 filter paper placed on a funnel vial. The filtrate is reserved for the tests.

Plant. Fresh or frozen minced plant tissue (2.5 grams), usually stems, is placed in a Waring Blendor and 100 ml. of extracting solution and 1 level teaspoonful of carbon are added. The mixture is agitated for 5 minutes and the pulp is filtered on a Whatman No. 1 filter paper. The filtrate is reserved for rapid tests (*5*). At the same time, 2.5 grams of the minced tissue are selected for moisture determinations.

Calibration of Standard Curves. Aliquots of the standard solutions are diluted with extracting solution to the designated level (Table I) and are then treated as in the determination of nutrients in soil and plant extracts. Photometer readings are taken using the appropriate filter and adjusting the blank to designated values (Table I). Deflection-concentration curves for each nutrient are drawn from the resultant data.

Determination of Nutrients in Soil and Plant Extracts. *Nitrate Nitrogen.* Either 5 or 2.5 ml. of soil or plant extracts (depending on the concentration present) are pipetted into a series of photometer tubes (previously marked at 25 ml.). The 2.5-ml. portion is diluted to 5 ml. with extracting solution and 1 ml. of 15 per cent sodium hydroxide solution is added to each tube. The contents are evaporated to dryness on the steam bath. The racks are removed, the tubes cooled, and 2 ml. of phenoldisulfonic acid are added to each. The salts are broken up quickly with a stirring rod, and are allowed to stand until cool. The contents are diluted to 25 ml. with 15 per cent sodium hydroxide, stirred, and allowed to cool to 35° C. Photometer readings are immediately taken using the 425 blue filter, and adjusting the null to give a reading of 100 with the blank.

Phosphorus. Four milliliters of ammonium molybdate and 2 ml. of aminonaphtholsulfonic acid are added to each tube containing 20 ml. of plant extract or 5 ml. of soil extract diluted to 20 ml. The contents are stirred. After 15 minutes, the contents are again stirred, and photometer readings are taken on the log scale using the 425 blue filter and a null adjustment to give a zero reading with the blank.

Potassium. One milliliter of soil or plant extract is brought to 10-ml. volume with extracting solution. The contents of the tubes are cooled to 10° C., and 1 ml. of cooled sodium cobaltinitrite solution is added to each. Ten milliliters of isopropyl alcohol solution are run in quickly down the sides of the tubes. The tubes are stoppered and after 2 minutes are rotated, slowly at first but increasing in speed until the contents are thoroughly mixed. After 5 minutes, photometer readings are taken using the 650 red filter and a null adjustment giving 100 per cent transmission with the blank.

Calcium. Four milliliters of sodium oxalate are added to each tube, containing 20 ml. of plant extract or 1 ml. of soil extract diluted to 20 ml. The contents are stirred, allowed to stand for 15 minutes, and restirred, and photometer readings are taken using the 425 blue filter and a null adjustment giving a reading of 100 with the blank.

Magnesium. One milliliter of soil or plant extract is diluted to 20 ml. with extracting solution, and 1 ml. of Titan yellow and 3.5 ml. of 15 per cent sodium hydroxide are added to each tube. The contents are mixed by means of a flat-bottomed rod. Readings are taken after 5 minutes using the 525 green filter and a null adjustment giving a reading of 90 per cent transmission with the blank.

Discussion of Methods

Selection and Preparation of Samples. *Soil.* The selection and preparation of soil samples have been fully discussed elsewhere (*7*, *8*, *12*).

Plant. The concentration of soluble nutrients in plants varies with the concentrations and relative proportions of nutrients in the soil, with prevailing climate, with the portion of the plant tested, and with age. It is important, therefore, that the latter two factors, which are controllable, be kept constant in order to enhance reliability. Until criteria are established for every nutrient and for every plant of all physiological ages, it is important that the concentration of test plants be compared with those of normal plants grown under the same climatic conditions.

Any portion of the plant can be used for testing. However, it is felt that the concentration of soluble nutrients in the older portions of the stem is the best general index of the supplying power of the soil (*2*). Concentrations in this portion of the plant are not usually excessive, and no large dilution errors are introduced (*14*). However, the portion

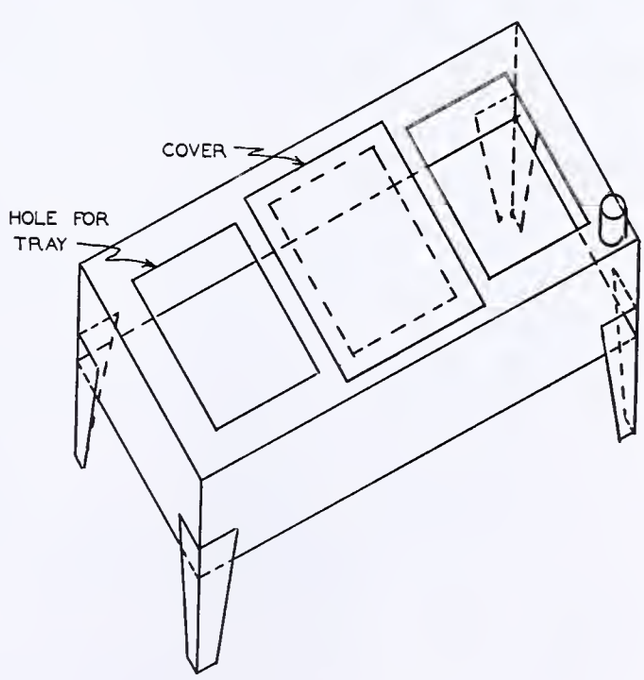

Figure 2. Water Bath

Table II. Recovery from Mixtures of Standard Solutions and of Soil and Plant Extracts Plus Standard Solutions

Substance	NO_3 Found	NO_3 Calcd.	NO_3 Diff.	P Found	P Calcd.	P Diff.	K Found	K Calcd.	K Diff.	Ca Found	Ca Calcd.	Ca Diff.	Mg Found	Mg Calcd.	Mg Diff.
	P. p. m.	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*
Mixed solutions	5.2	5.0	+0.2	4.9	5.0	−0.1	50.0	50.0	0	26.0	25.0	+ 1.0	26.6	25.0	+1.6
Soil extract	6.2	..	...	8.0	..	...	40.0	...	....	194.0	...	....	19.0	..	...
Soil extract + mixed solutions	27.5	28.2	−0.7	17.0	18.0	−1.0	150.0	140.0	+10.0	212.0	244.0	−32.0	36.2	34.5	+1.7
Plant extract	2.5	..	..	7.8	..	...	150.0	...	....	13.1	..	....	33.4	..	...
Plant extract + mixed solutions	40	37.5	+2.5	9.2	8.9	+0.3	175.0	175.0	0	34.0	31.5	+ 2.5	39.2	41.7	−2.5

Table III. Concentration of Soluble Nutrients in Soils and Plants as Determined by Rapid Tests[a]

Field No.	Soil NO_3-N	Soil P	Soil K	Soil Ca	Soil Mg	Lima Bean Stems NO_3-N	Lima Bean Stems P	Lima Bean Stems K	Lima Bean Stems Ca	Lima Bean Stems Mg	Yield No. of Pods[b]
	Pounds per acre					*P. p. m. of dried tissue*					
M-10	40	8.8	134	1720	209	4000	2270	1815	8400	6800	10
M-10	20	11.2	104	1800	209	3100	1470	1220	7000	6100	2
C-82	50	16.0	172	1168	272	3080	1490	2980	8760	6200	31
C-82	25	16.0	232	1064	252	2200	1550	2700	7650	7400	2
M-2	50	15.2	224	1456	145	3620	1320	1470	7240	5300	17
M-2	25	16.0	224	1456	145	2250	1885	1350	6510	5700	1

[a] As extracted by Morgan's Universal extracting solution.
[b] Per plant, average of 6 plants.

of the plant tested will depend to a certain extent on the type of plant and the purpose of the investigation. The various interrelationships of some of the nutrients in the plant and their possible importance in selection of samples have recently been discussed (*9*, *13*).

After collecting the samples, extracts should be prepared as soon as possible. These extracts, if placed in tightly stoppered flasks, will keep for several months without appreciable change. If impossible to prepare extracts immediately, the plants should be frozen without delay and kept in cold storage until time is available. The frozen material is then minced, weighed, thawed, and extracted as before.

Tests. A blank should be run with all determinations. In the final part of the nitrate analysis, samples should be cooled quickly and read immediately for best results.

The time consumed in running the nitrate nitrogen tests can be materially reduced by using three trays. The tubes in the second tray are prepared while the first tray is on the steam bath, etc. By the time the samples in the first tray have been tested, the tubes in the second tray will be almost ready for the additions of the phenoldisulfonic acid. Using such a system, it is a simple matter to determine quantitatively the nitrate nitrogen content of 72 samples in a day.

In the phosphorus determination, full color is not developed for several hours. However, comparative repeatable results can be obtained after 15 minutes.

The precipitate formed in the potassium determination may be of variable composition (*10*) and is influenced a great deal by temperature, rate of shaking, and ammonia present. The temperature of the solution at time of precipitation and the rate of shaking should be kept as uniform as possible. Concentrations of ammonia up to 100 p. p. m. in the extracts do not interfere, providing formaldehyde is used. Since the amounts of ammonia in the extracts are usually less than this amount, no correction for ammonia need be made.

In the calcium determination, the results are more accurate if the sodium oxalate reagent is prepared daily. The oxalate reagent undergoes changes in concentration upon standing, with a corresponding change in the calcium oxalate precipitate formed. Even with a fresh sodium oxalate reagent, it is desirable to stir the reagent thoroughly before adding it to a batch of extracts.

The determination of magnesium is complicated by the fact that the Titan yellow reaction is appreciably influenced by changes in pH and salt concentration. Plant and soil extracts add some buffering effects and salts. The errors are reduced to negligible factors by using small samples of extracts for analysis.

Calculations. Concentrations in parts per million can be read directly from the deflection-concentration curves or from charts prepared from such curves.

It is desirable to report soil nutrients in pounds per plowed acre (assuming 2,000,000 pounds of air-dry soil per acre to a plowed depth), and concentrations of plant nutrients in parts per million on a dry weight basis.

Accuracy. The methods proposed give results not entirely comparable to longer methods of analysis, but of definite value in determining relative differences between the soluble nutrients in soils and plants. Recoveries of added nutrients are good (Table II). Determinations can be repeated within 0.75 p. p. m. of nitrate nitrogen, 0.1 p. p. m. of phosphorus, 0.2 p. p. m. of potassium, 0.5 p. p. m. of calcium, and 0.1 p. p. m. of magnesium. Using the recommended aliquots, these variations would represent about 150 p. p. m. of nitrate nitrogen, 20 p. p. m. of phosphorus, 400 p. p. m. of potassium, 100 p. p. m. of calcium, and 400 p. p. m. of magnesium per dry weight of plant. For soils, it would represent 3 pounds of nitrate nitrogen, 1.6 pounds of phosphorus, 8 pounds of potash, 40 pounds of calcium, and 8 pounds of magnesium on an acre basis. In most cases, the above values represent an accuracy of ±10 per cent in duplicate analysis of the same extracts of one sample. Analysis made on duplicate samples of soil or plants will have greater variations. The variations in concentrations within samples from the same field are often associated with different types of growth (Table III).

Adaptations. The methods presented are based on the use of a Fisher electrophotometer and of Morgan's Universal extracting solution. They may readily be adapted to other photoelectric colorimeters and other types of extracting solutions.

Conclusions

Rapid methods for the determination of soluble nutrients in soils and plants are presented. A photoelectric colorimeter is used to determine accurately concentrations of nutrients in separate aliquots of extracts. Only one extract of either soil or plant is made, using one extracting solution for all determinations. Determinations on the same extract in most cases can easily be repeated within ±10 per cent.

Acknowledgment

The author wishes to express his gratitude to Frank App, director of the Research Division, for his many suggestions and to Raymond Webster for the drawings.

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Photometric Determination of Benzene, Toluene, and Their Nitro Derivatives

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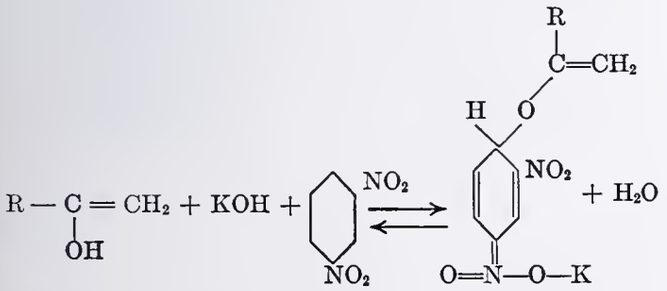
Certain factors relating to the production of maximum color from nitro compounds with butanone and alkali have been studied and modifications of existing procedures are recommended. The analysis of mixtures of benzene and toluene, such as occur in some new commercial solvents, has been improved by introducing a differential oxidation of the nitro derivatives.

THE fundamental reaction between certain nitro compounds and ketones in the presence of concentrated alkali has been studied by many workers (*1, 2, 4–7, 9, 11*). A summary of their findings leads to the following conclusions:

Mononitro derivatives of benzene and its homologs do not give the reaction. Among the dinitro derivatives tested, only those of the meta series (nitro groups meta to each other) respond. When further substitution in the ring occurs, only those compounds retaining ketone solubility give color. At least one position para to one of the nitro groups must be unsubstituted. Blues or reds signify the presence of aromatic polynitro derivatives of this class. Certain substitutions, such as hydroxyl and amino groups, change these colors to yellows and oranges and indicate a different reaction. The concentration of alkali used determines the intensity of the color obtained and the rapidity of its production.

The evidence indicates a condensation between the enol form of the ketone and the aci-nitro form of the nitro compound with the elimination of a molecule of water:



The colors gradually fade, and brown insoluble compounds are produced (*11*). In some cases, one of the nitro groups is replaced (*13*).

Quantitative procedures have been described by Pearce, Schrenk, and Yant (*10, 12, 14, 15*) and by Kay (*8*) but are not entirely satisfactory regarding the strength of alkali used or the development and measurement of the colored compounds produced. The analysis of mixtures of benzene and toluene was only approximate (*14*). The present report describes some improvements which were made in these procedures.

Experimental

Reagents. Fuming nitric acid, specific gravity 1.50; chromic acid (CrO_3), saturated aqueous solution; potassium hydroxide, 70 per cent (one pound of pellets, dissolved and diluted to 650 ml.); and butanone.

Apparatus. Nitration of hydrocarbons is carried out in a small U-tube filled with beads. The sample is transferred to the tube from a gas buret by means of mercury if vapors are being analyzed, or, if the hydrocarbons are in aqueous solution, they may be blown over by means of a current of air. Rubber connections and greased stopcocks may not be used. A suitable lubricant for stopcocks may be made from starch and glycerol (*3*).

A Bausch & Lomb visual spectrophotometer with 5- and 10-mm. cells was used, although any other suitable instrument could be substituted.

Procedure. The sample containing 10 to 100 micrograms of a mixture of hydrocarbons is collected in 2.0 ml. of fuming nitric acid in the nitration tube filled with beads. The tube and contents are heated in an oil bath at 90° C. for 15 minutes. If benzene only is to be determined, 1 drop of a saturated chromic acid solution is added and the sample is mixed by bubbling a little air through it. If benzene, toluene, and xylene are to be determined, the chromic acid is omitted.

The tube and sample are returned to the oil bath for another period of 15 minutes.

Beads and sample are transferred to a 60-ml. glass-stoppered bottle with the aid of three 2.0-ml. portions of water. The mixture is cooled in ice water and carefully neutralized with 70 per cent potassium hydroxide until the dichromate orange changes to chromate yellow-green. In the toluene determination, where no oxidation is required, 1 drop of chromic acid solution may be added after dilution and cooling without loss of toluene.

Ten milliliters of butanone are added to the neutralized sample, and the bottle is firmly stoppered and shaken vigorously. It is placed in a water bath at 60° C. to prevent crystallization of potassium nitrate and shaken occasionally during 5 to 10 minutes.

The sample is poured into a jacketed buret kept at about 60° C. and allowed to separate. The beads are caught in a funnel which is fitted with a wire to prevent closure of the stem by the beads.

The lower aqueous layer is carefully withdrawn and discarded and the upper butanone layer containing the nitrated sample is drained into a 25-ml. glass-stoppered cylinder. Ten milliliters of 70 per cent potassium hydroxide are added and the mixture is shaken vigorously for 2 minutes. A motor-driven shaker has been found convenient if a large number of analyses are required.

The colored butanone layer is allowed to separate and is transferred to the cell of the photometer.

If the color is too dark for satisfactory measurement, the butanone may be quantitatively diluted or a shorter cell may be

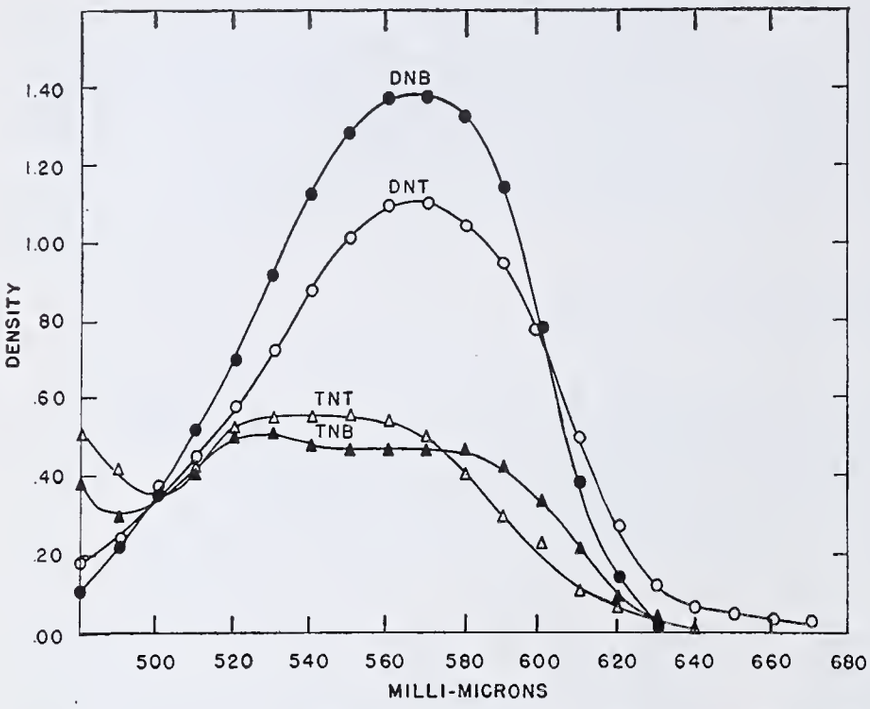


FIGURE 1. SPECTRAL ABSORPTION CURVES OF BUTANONE-NITRO COMPOUNDS

DNB. *m*-Dinitrobenzene *TNB.* 1,3,5-Trinitrobenzene
DNT. 2,4-Dinitrotoluene *TNT.* 2,4,6-Trinitrotoluene

substituted. Owing to fading of the colors, not more than 5 to 10 minutes should elapse between the shaking of the mixture and the reading of the instrument.

A calibration curve should be prepared relating milligrams of hydrocarbon to color density for each substance determined, and since losses occur in the several steps, standards must be treated exactly as the samples are treated. Color density = $\log \frac{1}{\text{transmission}}$.

Since the density-milligram curve is linear, it is convenient to express the relation by a factor.

Table I gives the factors calculated when nitro compounds and hydrocarbons are analyzed by the procedures described. They are expressed as density per milligram of hydrocarbon for a 10-mm. cell. The corrected values are obtained by making allowances for loss of butanone in extraction and loss of water in dehydration. Comparison of these corrected values with those obtained with dry butanone standards gives a measure of the losses of nitro compound sustained in nitration and extraction. The small increase shown upon heating nitric acid solutions of dinitrobenzene indicates that traces of mononitrobenzene are present in the preparation. The dinitrotoluene sample is purer, as judged by the identity of the values for heated and unheated samples.

Some interesting comparisons regarding the nitration of benzene and toluene can be made. The values obtained indicate that 86 per cent of benzene and 76 per cent of toluene are converted to *m*-dinitro compounds by fuming nitric acid when liquid hydrocarbons are used. The corresponding figures for the vapors are 77 and 78. The losses may be due to the formation of ortho and para isomers and to oxidation. That no appreciable loss was due to inefficient trapping of vapors by the nitration tube was shown by introducing a second tube in series with the first.

ABSORPTION CURVES. Figure 1 shows the absorption curves for several of the compounds studied. The wave length selected for quantitative comparison was 560 millimicrons.

PREPARATION OF BUTANONE EXTRACTS. It is not possible to develop these colors quantitatively in the presence of water or potassium nitrate (*14*); therefore, the nitro compounds formed during nitration of aromatic compounds must be extracted from the nitration mixture. Butanone is fairly soluble in acid solutions, and nitro compounds are only partially extracted from alkaline solutions of pH greater than 10.

The most convenient indicator to use for adjusting the pH for extraction is the chromic acid solution used as oxidant in differentiating benzene from toluene. A sharp change from orange to yellow-green occurs at about pH 7.

Factors Affecting Color Development

According to the mechanism of the reaction proposed, the alkali serves several functions: converts ketone to enol form, converts nitro compound to aci-nitro salt, and removes water formed by condensation. Figure 2 shows the effect of increasing concentration of alkali on density of color obtained from nitrated benzene and toluene. Owing to losses of butanone during extraction without corresponding losses of nitro compound, the densities were too high. A correction was therefore made and these values were plotted. The increase in density obtained by increasing concentrations of alkali is best explained as an increase in efficiency of the alkali as a dehydrating agent. Almost the maximum density is obtained with 50 per cent alkali and dinitrobenzene, whereas only about one third of the maximum density is obtained with dinitrotoluene and this concentration of alkali.

Further confirmation of the dehydrating action of the alkali is shown in Figure 3, where the larger volumes of 70 per cent alkali solution give greater color densities than the smaller volumes.

Potassium nitrate inhibits color development, as shown in Figure 4. Seventy per cent potassium hydroxide solution was saturated with dry potassium nitrate and the resulting

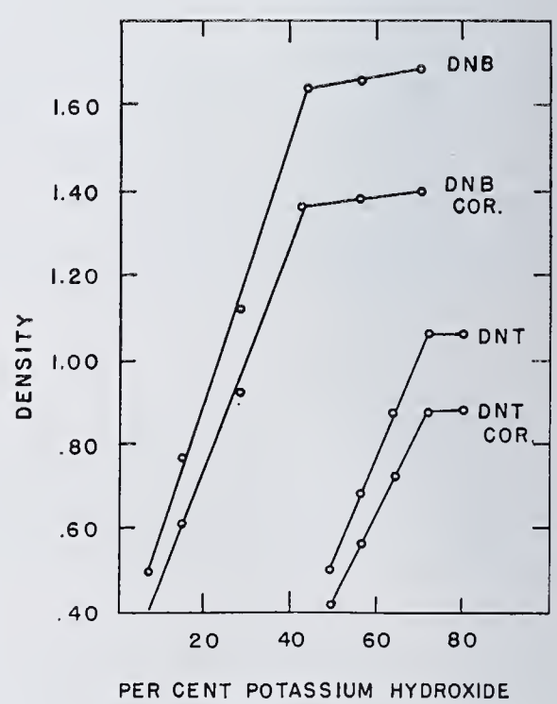


FIGURE 2. COLOR DENSITY AT VARIOUS CONCENTRATIONS OF POTASSIUM HYDROXIDE

Table I. Color Density per Milligram of Hydrocarbon

Standards	Benzene		Toluene	
		After CrO_3		After CrO_3
Butanone + *m*-dinitro	30.3 ± 0.7	...	20.9 ± 0.4	...
HNO_3 + *m*-dinitro (unheated)	32.1 ± 0.8	...	24.0 ± 0.9	...
HNO_3 + *m*-dinitro (corrected)	26.3 ± 0.7	...	19.7 ± 0.7	...
HNO_3 + *m*-dinitro (heated)	35.2 ± 1.4	33.4 ± 1.6	23.8 ± 0.9	0.0
HNO_3 + *m*-dinitro (corrected)	28.8 ± 1.1	27.4 ± 1.3	19.5 ± 0.7	0.0
HNO_3 + liquid (heated)	30.1 ± 0.5	28.8 ± 1.2	18.2 ± 1.4	0.0
HNO_3 + liquid (corrected)	24.7 ± 0.4	23.6 ± 1.0	14.9 ± 1.2	0.0
HNO_3 + vapor (heated)	27.0 ± 1.6	27.6 ± 2.0	17.4 ± 0.5	0.0
HNO_3 + vapor (corrected)	22.1 ± 1.3	22.6 ± 1.6	14.3 ± 0.4	0.0
Butanone + trinitro	8.8 ± 0.3	...	9.6 ± 0.4	...

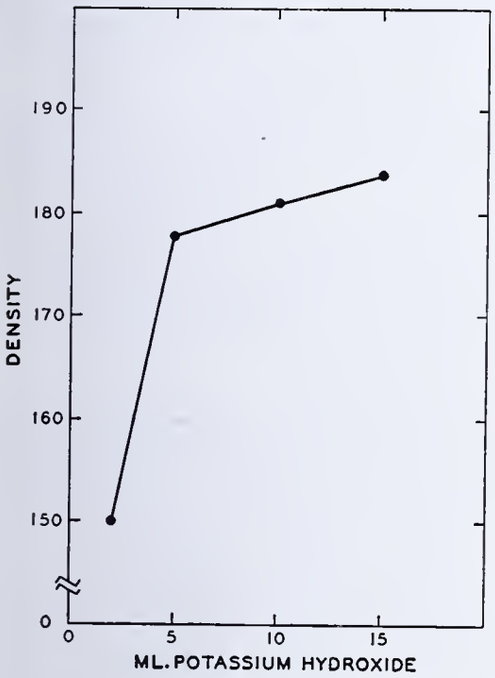


Figure 3. Color Density and Volume of 70 Per Cent Potassium Hydroxide

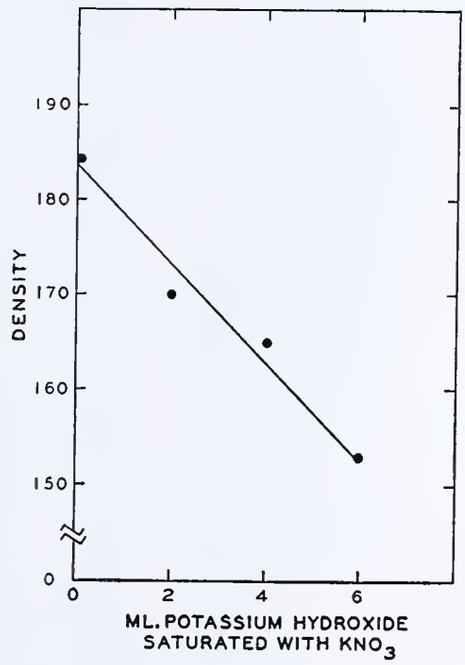


Figure 4. Effect of Potassium Nitrate on Color Density

solution mixed in various proportions with 70 per cent potassium hydroxide solution. Ten milliliters of these mixtures were shaken with the butanone extract of nitrated toluene and the densities determined.

This experiment emphasizes the importance of making a clean separation of the butanone extract from the aqueous phase in the determinations. It also supports the need for larger volumes of potassium hydroxide solution used to develop the colors than were recommended by Pearce *et al.* (*10*).

Differentiation of Benzene and Toluene

When mixtures of benzene and toluene are nitrated with fuming nitric acid, heated, diluted, neutralized, and extracted with butanone, the density obtained is equal to the sum of those obtained with the individual substances separately. If, however, the mixture is oxidized with chromic acid after nitration, only the color of the benzene derivative is obtained.

A paired series of mixtures was prepared by dissolving various amounts of standard benzene and toluene solutions in 2.0 ml. of fuming nitric acid. One set of mixtures received 1 drop of a saturated aqueous solution of chromic acid in each sample; the other set served as controls. Both sets were placed in an oil bath at 90° C. for 15 minutes and then diluted, neutralized, and extracted as usual. The results in Table II show an average error of ±5.0 per cent.

Table II. Analysis of Benzene and Toluene Mixtures

Sample	Taken		Found	
	Benzene	Toluene	Benzene	Toluene
	Micrograms		*Micrograms*	
1	71.5	00.0	71.9	00.0
2	57.2	15.7	59.2	15.7
3	42.9	31.4	42.8	34.6
4	28.6	47.1	27.4	48.1
5	14.3	62.8	13.7	65.0
6	00.0	78.5	2.3	73.3

Analyses of benzene and toluene vapors and of mixed solvents have shown the methods to be equally satisfactory.

The differentiation probably depends upon the loss of butanone solubility of the oxidized products. Experiments with 2,4-dinitrobenzoic acid indicate that dinitrotoluene is oxidized to this substance. *m*-Dinitrobenzene is not oxidized under the conditions selected but is lost with permanganate oxidation and with longer heating periods with the oxidant chosen.

Summary

The colored compounds formed by shaking *m*-dinitrobenzene and its homologs with ketones and alkali are probably quinoids formed by condensation of the aci-nitro and enol forms with the elimination of water.

Various factors concerned with the production of these colors have been studied, and certain modifications recommended for their quantitative determination. The use of 70 instead of 50 per cent alkali greatly hastens and increases color production.

Mixtures of benzene and toluene have been satisfactorily analyzed by oxidation of dinitrotoluene, presumably to dinitrobenzoic acid, which gives no color under the conditions chosen. *m*-Dinitrobenzene is not attacked and gives its usual color with undiminished intensity.

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Colorimetric Determination of Alkyl Benzene Sulfonates

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Simple colorimetric quantitative method for the estimation of alkyl benzene sodium sulfonates is described which is sensitive to one part per million of pure sulfonate. The analysis may be made visually or photometrically.

METHODS for the determination of the active ingredient content of commercial surface-active agents, including alkyl benzene sulfonates, have been the subject of several papers (*3*, *4*, *5*). Few of these methods are entirely satisfactory, because they include impurities such as sodium sulfate, or are inoperative with dilute solutions of surface-active agents. The determination of surface tension depression is easily accomplished, but does not lend itself to strictly quantitative estimation.

The need for a rapid method for the quantitative estimation of alkyl benzene sodium sulfonates in dairy cleansing processes is described by Scales and Kemp (*6*), who outline a qualitative test based upon the formation of a blue coloration when a solution of alkyl benzene sulfonate is added to a solution of *o*-tolidine and sodium hypochlorite. However, not all detergents undergo this reaction, since they found that soap, Turkey red oil, lauryl sodium sulfate, and three related compounds failed to produce the iodo-blue color characteristic of this test. Under their conditions a flocculent precipitate obscured the test, so that it was not considered sufficiently accurate for quantitative purposes.

Re-examination of their data and further investigation proved that the test could be made quantitative in character.

Visual Method

EQUIPMENT. 50-ml. Nessler tubes (or 50-ml. graduates), pipets, and a 10.0-ml. buret graduated in 0.05-ml. divisions.

SPECIAL SOLUTIONS. *o*-Tolidine Solution. Add 1.0 gram of *o*-tolidine to 5 ml. of 20 per cent hydrochloric acid (100 ml. of hydrochloric acid, specific gravity 1.18–1.19, to 500 ml. of distilled water) and grind to a thin paste. Add 150 to 200 ml. of distilled water to produce a solution. Transfer to a 1-liter graduate and make up to 505 ml. with distilled water, then make to 1 liter by adding the balance of the 20 per cent hydrochloric acid solution. Store in an amber bottle out of direct sunlight.

Sodium Hypochlorite. Prepare a solution of sodium hypochlorite by slurrying 5 grams of high test calcium hypochlorite and 4 grams of anhydrous sodium carbonate in 334 ml. of distilled water. Let the insoluble matter settle, then decant the solution through a filter. Analyze it for available chlorine content according to the A. O. A. C. (*2*) arsenious oxide titration method. Prepare a standard solution containing 300 p. p. m. of available chlorine.

Alkyl Benzene Sodium Sulfonate, or Unknown. Prepare a 0.05 per cent solution of the alkyl benzene sulfonate by suitably diluting a stronger solution with distilled water. Dilute a wash solution 1 to 5 or more, depending upon the approximate concentration of the alkyl benzene sulfonate. This solution must be as dilute as indicated, or if stronger must be measured accurately from a suitably calibrated buret or pipet.

PROCEDURE. Transfer 40 ml. of distilled water to each of two Nessler tubes. Add 1 ml. of 300 p. p. m. sodium hypochlorite solution to each tube, and invert to mix. Add 2 ml. of *o*-tolidine solution to each, and invert to mix.

Make the blank up to 50 ml. with distilled water and invert several times to mix thoroughly.

Add the unknown solution of alkyl benzene sulfonate of definite dilution carefully in known amounts to a fresh mixture of reagents, until a color change just appears. Make the tube up to volume and compare with the blank. From the volume of unknown added, and the fact that 1 p. p. m. of alkyl benzene sodium sulfonate is required for a color change, calculate the strength of solution.

Used in the manner described above, the method is suitable for determining the strength of dilute solutions by visual observation where photometric equipment is not available.

Experimental

The experimental information developed for the visual method is applicable to use in photometric equipment. The several variable factors involved were investigated.

Benzidine and diamidine were tested to find whether they would produce a more suitable color for analytical purposes, but *o*-tolidine produced the most satisfactory results.

Tests were made to determine the effect of quantities of reagents upon the color formation.

To show the effect of available chlorine, a standardized solution of 300 p. p. m. of sodium hypochlorite was used, and known increments were added to 50-ml. Nessler tubes containing 30 ml. of distilled water, 5 ml. of *o*-tolidine solution, and 0.5 ml. of 1 per cent alkyl benzene sodium sulfonate solution. The volume was made up to 50 ml. and the colored solutions were examined. The results, shown in Table I, indicate optimum color formation at 6 p. p. m. of available chlorine in the final test solution.

To determine the optimum amount of *o*-tolidine solution, 1-ml. increments of *o*-tolidine solution were added to aqueous solutions containing 300 p. p. m. of available chlorine and 0.5 ml. of a 1 per cent solution of alkyl benzene sulfonate. The 1-ml. increment produced a brownish blue coloration, with very little precipitate; 2 ml. gave a deep blue; and increasing amounts of *o*-tolidine produced a greenish blue color with a greater amount of precipitate.

The order of addition of reactants had a profound effect upon the formation of the undesirable surface precipitate. Addition of the alkyl benzene sulfonate solution just before making up to volume minimized this difficulty.

TABLE I. OPTIMUM AMOUNT OF AVAILABLE CHLORINE

(Varying increments of NaOCl solution, 5.0 ml. of *o*-tolidine solution, 0.5 ml. of 1% alkyl benzene sulfonate solution, q. s. water)

No Chlorine (Control)	Available Chlorine in Test Solution			
	1 p. p. m.	3 p. p. m.	6 p. p. m.	24 p. p. m.
Yellow solution	Yellow precipitate	Blue solution, precipitate on surface	Deep blue solution, precipitate on surface	Brownish blue solution, large amount of precipitate on surface

Utilizing the optimum amounts of available chlorine (300 p. p. m.) and *o*-tolidine solution (2 ml.), the desired dilution of alkyl benzene sulfonate was ascertained with varying amounts of pure alkyl benzene sulfonate solution: 0.0005 gram of sulfonate produced a suitable coloration, whereas 0.004 gram resulted in a deep brown color. This indicated that somewhat less than 80 p. p. m. of alkyl benzene sulfonate should be present in the test solution.

Time (up to 15 minutes) had no effect upon the end point, but it is preferable to make the test shortly after all the ingredients have been mixed.

Tap water (ca. 300 p. p. m. hardness) produced no visible effect.

Table II. Examination of Solution for Optimum Wave Length and Transmittance

Wave Length *Millimicrons*	Transmittance %
500	8.8
600	0.3
700	17.0
800	13.0
900	21.5
1000	16.0

Alkalies in wash solutions have little effect upon the test under the conditions observed—i. e., if the alkyl benzene sulfonate solution is alkaline, the degree of dilution will be such that final pH will be adjusted by the *o*-tolidine solution.

A color change is produced by as little as 0.00005 gram of pure sulfonate in 50 ml. of solution according to test. This is equivalent to 1 part per million. Alkyl benzene sodium sulfonates diluted with sodium sulfate are detected in proportionally small amounts, dependent upon the degree of dilution of the active ingredient.

Table III. Photometric Transmittance Data

(Sample A, 40% alkyl benzene sodium sulfonate; sample B, 100% alkyl benzene sodium sulfonate; sample C, 100% *n*-butyl diphenyl sodium sulfonate)

Parts per Million	Sample A Photelometer B filter	Sample A Spectrophotometer, 600 mμ	Sample B Photelometer B filter	Sample B Spectrophotometer 525 mμ	Sample B Spectrophotometer 600 mμ	Sample C Photelometer B filter
0.25	..	..	97	99	97	..
0.5	96.5	97	97	98	95.5	95
1.0	96.5	96	94.5	96	92	94
1.5	97	95	91.5	92	87	93
2.0	96	94	87.5	88	84	94
2.5	95	95	85	84	78	93
3.0	92	94	80.5	80.5	73.5	92.5
3.5	93	93	77.5	78	70.5	93
4.0	91	91	74.5	74.5	66.5	91.5
4.5	..	91	69	71.5	63	..
5.0	89	88	69	68	59.5	91
6.0	84.5	84.5	60.5	..	52.5	..
7.5	82.5	81	56.5	..	42.5	89
10.0	75.5	73.5	43.5	44	32.5	..
15.0	65	60	32	28	18.0	81
20.0	56	48	24	19	11.5	79
25.0	47	38.5	17	13	6.0	71
30.0	45	30	12	8.5	3.5	66
40.0	33	20.5		4.5	1.7	58
50.0	27	14.5				54

Photometric Method

The analytical procedure used is exactly the same as that for visual observation. In this case, however, a suitable filter must be chosen with instruments such as the Cenco-Sheard-Sanford photelometer, or a wave length of maximum color absorption with instruments such as the Coleman spectrophotometer. Once this factor has been ascertained by examination of the blank and of a test portion which contains the maximum expected amount of alkyl benzene sulfonate, the problem is to prepare sufficient data to graph percentage transmittance *vs.* concentration. If such a curve substantially conforms to Beer's law, it may be used as reference for samples of unknown concentration.

A filter which produced satisfactory results with the Cenco-Sheard-Sanford photelometer was the B filter with maximum transmission at 525 millimicrons. Examination of the solutions with the Coleman spectrophotometer indicated that satisfactory results could be obtained in a range lying between 500 and 600 millimicrons.

Typical data developed by this method of analysis are shown in Table III. The data for sample B at 525 millimicrons and with the B filter are plotted in Figure 1. The curves closely approach straight lines, hence conform to Beer's law.

Solutions of alkyl benzene sulfonates of unknown strength can be prepared for test as described in the procedure, and then submitted to photometric analysis. From the percentage transmittance of such test solutions, the concentration may be ascertained by reference to the curve. The concentration of active alkyl benzene sulfonate in the original sample of unknown strength can be calculated from the size of the sample used in the test, and its degree of dilution. It is suggested that such a dilution of unknown be chosen that an appreciable coloration be formed, and obviously, that it fall within the limits of the curve.

Tests were made with sodium sulfate-containing products which were calculated to contain 40 ± 1 per cent of active ingredient as alkyl benzene sodium sulfonate.

A more or less usual method for determining the active ingredient is to extract a 1.00-gram sample of the product with 200 ml. of neutral 95 per cent alcohol in a 400-ml. beaker on the water bath, for at least an hour, replacing the alcohol which evaporates. The residue is then quantitatively transferred to a tared Gooch crucible, washed with 100 ml. of boiling alcohol, and dried at 105° C. to constant weight.

The moisture content of the sample was determined by the xylene distillation method (*1*). The active ingredient content was determined by difference: 100 minus the sum of the alcohol-insoluble plus moisture yielding the active ingredient content.

It is recognized that the alcohol-insoluble method will yield low results because of the difficulty of removing all the alcohol-soluble active ingredient from the residual sodium sulfate. The results with three samples are shown in Table IV.

The photometric method was used for analyzing the same samples by adding 5 ml. of a 0.05 per cent (dry basis) solution of the sample to the mixture of reagents described in the procedure. The percentage transmittance of the unknowns was measured with a B filter using the photelometer. The per-

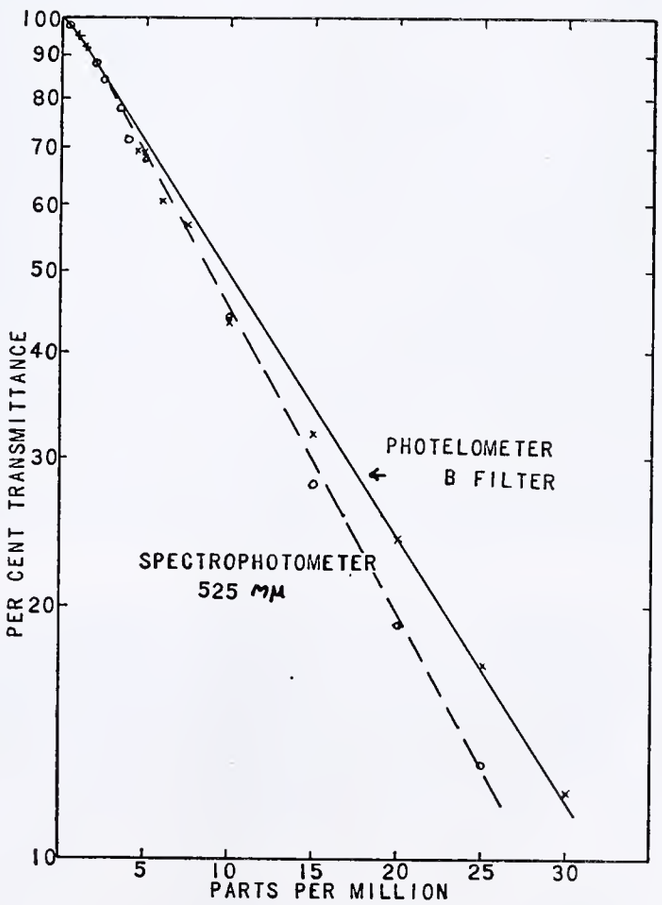

Figure 1. Typical Transmittance-Conductance Curve for Pure Alkyl Benzene Sulfonate (Sample B)

Table IV. Comparison of Gravimetric Alcohol-Insoluble Method with Photometric Method

Sample No.	Moisture (Xylene Distillation)	Alcohol Insoluble	Active From alcohol-insoluble method by difference	Active From photelometer B filter, sample B
	%	%	%	%
1	5.0	59.25	35.75	41.0
2	4.0	59.07	36.93	39.6
3	6.0	60.05	33.95	38.6

centage of active ingredient for each of the samples was calculated from the concentration of the sample, and reference to the photelometer curve for sample B, Figure 1. This information is shown in Table IV. It is obvious from this that the photometric method gives more nearly correct results than does the alcohol-insoluble method.

There was a possibility that certain other alkyl aryl sulfonates might be amenable to this method of estimation. Tests were made with two alkylhydroxydiphenyl sulfonates and *n*-butyldiphenyl sodium sulfonate (Table III). Apparently the hydroxy group interferes with this particular color formation, since only the latter compound produced a color approaching the characteristic iodo-blue. Improved characteristics of this curve could have been obtained by using a more suitable wave length for examination of the test solutions. This method of analysis may be suitable for other alkyl aryl sulfonates.

Acknowledgment

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Identification of Carbon Black by Surface Area Measurements

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PARKINSON (*6*) has suggested that the particle size of lampblack recovered from a tire tread stock by means of nitric acid is unchanged from that of the original supply. His conclusions were drawn from sedimentation experiments. The authors have found (*8*) that the low-temperature nitrogen-adsorption method of Emmett and his co-workers (*1*, *2*, *3*) for determining surface areas of finely divided substances is a comparatively rapid and accurate method of measuring the total surface of commercial carbon blacks. The object of the present investigation was to expand Parkinson's observation to a variety of commercial carbon blacks, employing the nitrogen-adsorption technique. Since the surface area of most of the standard carbon blacks has been previously determined (*3*, *8*), a method of identifying the carbon black present in an unknown rubber stock can be readily devised if it is first established that the carbon black can be recovered from a rubber stock with unchanged surface area.

The present paper reports the surface area values determined by the nitrogen-adsorption method for a number of commercial carbon blacks recovered from vulcanized tread stocks.

Experimental Details

The technique and apparatus for determining the surface area of carbon black by the low-temperature nitrogen-adsorption isotherm have been described in a previous publication (*8*). An oxygen thermometer is now used to measure the temperature of the liquid nitrogen bath during a run. From this temperature, the appropriate saturation pressure, p_0, of the adsorbate nitrogen is obtained and employed in calculating the final surface area value (*3*).

The carbon blacks studied were compounded in the following recipe:

Ingredient	Parts by Weight	Ingredient	Parts by Weight
Smoked sheet	100	Captax	0.9
Zinc oxide	5	Stearic acid	4.0
Sulfur	3	Agerite Hypar	1.0
Pine tar	3	Black	45

One stock was made from Grade 6 carbon black in Buna S:

Buna S	100
Zinc oxide	5
Sulfur	2
Pine tar	2
B-L-E (antioxidant)	1.0
B-J-F (accelerator)	1.5
Laurex	2
Grade 6 black	50

The smoked sheet stocks were cured for 30, 60, and 90 minutes at 134° C., and the Buna S stock at 144° C. The 60-minute cure was nearly optimum in the majority of cases, and was the only cure selected for study.

The following commercial carbon blacks were selected for study:

Gastex, a nonimpingement-type black manufactured from natural gas. A semireinforcing black of the "soft" type widely used in rubber goods.

Acetylene Black (Shawinigan), prepared by thermal decomposition of acetylene. A soft-type black which does not possess the marked rubber-reinforcing properties of channel blacks. Its electrical conductance in rubber stocks is very high.

Spheron, grades 9 through 1, a series of rubber-reinforcing carbon blacks prepared by impinging natural gas flames on metal surfaces. Their essential difference is particle size, grade 9 being the coarsest and grade 1 the finest of these typical rubber blacks.

Spheron N, a channel black of fine particle size with superior electrical conducting properties when compounded in rubber stocks.

The free carbon in the vulcanized rubber stocks was separated by means of nitric acid. The procedure followed was essentially that of Oldham and Harrison (*5*).

In the authors' laboratory it is the practice to employ a 0.75- to 1.00-gram sample of rubber stock instead of the 0.15-gram sample suggested by Oldham and Harrison (*5*). While larger samples increase the time required for washing and filtering, it is the authors' experience that greater accuracy and reproducibility are attained thereby. Jacob Gabry, to whom the

Table I. Experimental Results

Black	Original Area	Area after Treating with HNO_3	Area after Recovery from Rubber By HNO_3	By cresol	Carbon in Rubber HNO_3 method	Cresol method	Carbon Calculated
	Sq. m./g.	*Sq. m./g.*	*Sq. m./g.*	*Sq. m./g.*	%[b]	%	%
Gastex	26[a]	...	22	21	28.4	27.7	27.8
Acetylene	64	67	63	70	..	..	..
Cabot grade 9	99	...	100	99	27.8	27.2	27.8
Cabot grade 6	109	112	110	107	27.7	27.4	27.8
Cabot grade 4	143	181	175	125	27.9	27.7	27.8
Cabot grade 1	210	...	203	156	28.1	27.7	27.8
Spheron N	330	316	326	204	..	..	..
Grade 6 in Buna S	109	...	113	...[c]	29.9	..	30.3

[a] A previous publication (*8*) reported 40 sq. m. per gram as the surface of standard Gastex. C. W. Snow, General Atlas Co., has kindly stated that this material was actually CS-3 carbon. The present value is that of standard Gastex.
[b] Ash-free basis.
[c] Not completely soluble.

authors are indebted for the analytical results reported here, also points out that it is essential to control the time of digestion of the rubber stock and nitric acid to 3 to 4 hours at the specified temperature of 60° to 70° C. While in the case of channel blacks, the weight of carbon black recovered by this method is generally reported (*5*) to be 105 per cent of its original weight, under the conditions reported here a value of 108 per cent has been found more general. Consequently, a correction factor of 1.08 is employed instead of the value 1.05 recommended by Oldham and Harrison (*5*).

One to 0.75 gram of the vulcanized stocks was digested for one hour with 15 cc. of concentrated acid. After the addition of another 35 cc. of acid, digestion was continued for 2 hours more. In order to avoid contamination of the black for the subsequent surface area determination, a Selas sintered filter crucible FS-20-100 was used in place of the usual asbestos Gooch. The crucible and black were washed according to the standard method, then dried at 110° for 2 hours. The per cent carbon in the rubber stock was determined directly without ignition. The surface area of the dried sample of recovered black was then determined by low-temperature nitrogen adsorption. The ash was determined on a duplicate sample by ignition. In all compounds studied, it amounted to about 0.4 per cent by weight of the whole rubber stock. Thus the recovered black as weighed out for the surface area determination contained 1.44 per cent ash. The surface values in column 4 of Table I are accordingly 1.44 per cent lower than if they had been calculated on ash-free basis. However, in the absence of data on the surface area of ash itself, such a simple correction is not justified. The correction in any case would not amount to more than 1 or 2 square meters per gram, a value too small to cause any confusion in the identification of the black.

It was necessary to establish that no appreciable change in surface area of the carbon black was brought about by the nitric acid during the separation of the black from the rubber stock. In order to establish this, "blanks" of 0.5-gram samples of carbon black alone were subjected to the same treatment employed with the compounded rubber stock and the surface areas of the blacks were then determined.

The use of cresol for the separation of carbon black from rubber stocks has been described by Roberts (*7*). In natural rubber stocks, this method gives excellent results for free carbon. It was felt that there would be less opportunity for alteration of the carbon surface during separation by this method than with concentrated nitric acid. The carbon black was separated from the stocks by cresol according to the method of Roberts (*7*). The surface area of the recovered carbon was then measured.

A single determination of the area of a grade 6 carbon black recovered from a Buna S stock with nitric acid was carried out. As it was not possible to get complete solution of this stock in cresol, the method had to be abandoned in this instance.

Experimental Results

Table I presents a summary of the data collected. With the exception of the Gastex and grade 9 channel black, which are values for single recoveries, the surface area values reported are the average of at least two independent recoveries. The deviation in all cases was not more than 3 per cent.

The data indicate that carbon black undergoes no appreciable alteration in surface area during incorporation in, or vulcanization of, a rubber stock. With but a single exception, the surface areas of the carbon blacks studied are substantially the same before incorporation and after removal from the rubber stock by means of nitric acid.

It is not possible to offer any ready explanation for the 25 per cent increase in surface of the grade 4 sample. This is not due to any peculiar effect of the rubber on the carbon black, since the black alone in nitric acid showed an appreciable increase in surface. There was nothing peculiar in the method of manufacture or properties of this particular sample to account for its apparent increased activity toward nitric acid. In any case, the alteration in the surface area of the recovered carbon is still not sufficient to confuse its identity.

In general, both the nitric acid and cresol methods gave excellent results for the amount of free carbon in the stocks. The surface areas of the blacks recovered by the cresol method show satisfactory agreement with the original supply in the case of the coarser blacks. However, with the finer particle blacks, the surface is considerably reduced. Thus, grade 1 black has an original surface of 210 square meters per gram. After recovery from the tread stock, by cresol extraction this value was only 156. After treating a 0.5-gram sample of this type black with cresol according to the standard procedure, the surface was 165 square meters per gram. Evidently a small amount of material is retained by the carbon in spite of the extensive washings and heating described in the procedure. The amount is not sufficient to alter the weight of the sample appreciably, but it is sufficient to block off certain interspaces in the black that were formerly accessible to the nitrogen molecule. Such an effect should become more pronounced the finer the particle size of the black. Accordingly, the cresol method of recovery is not satisfactory for separating carbon black from "unknown" rubber stocks for identification by surface area measurements.

The Buna S stock showed little solubility in cresol and quantitative recovery of carbon was not possible. It was possible, however, to get a satisfactory recovery of the carbon from the Buna S stock with nitric acid. In order to obtain complete solution, it was necessary to digest this stock with nitric acid somewhat longer than the specified 3 hours. This more drastic procedure is reflected in the slight increase in surface area of the recovered carbon.

Conclusions

In general then, we may conclude that carbon black can be recovered from rubber stocks with unchanged surface area. The nitric acid technique is the most effective method of effecting the separation. The digestion temperature must be controlled to between 60° and 70° C. for a total of not more than 3 to 4 hours. This method of identifying the carbon black in an unknown rubber stock is directly applicable only in the presence of a single type of carbon black. If blends of blacks are employed in the material under investigation, some secondary identification is also required. The non-impingement-type blacks, for example, are readily identified

by microscopic observation. From the known surface areas of these materials and the total per cent carbon present in the stock, a fairly positive identification of the blend can be made.

The fact that carbon black can be recovered quantitatively and with unchanged surface area from vulcanized rubber stocks appears to lend impetus to a physical concept of carbon black reinforcement. This point of view implies that any chemical combination between the ingredients of the rubber stock and the carbon black would be evidenced by some alteration in the surface of the latter. A few experiments were performed in an attempt to establish to what extent this concept was valid.

One hundred grams of grade 6 carbon black were intimately mixed with 6.6 grams of sulfur. This is about the ratio in which they are present in a standard rubber batch. Samples of this mixture were subjected to the standard curing temperature of 134° C. for 30, 60, and 90 minutes. The free sulfur was then extracted for 40 hours with acetone and the combined sulfur on the carbon was determined. Values of 0.16, 0.21, and 0.4 per cent combined sulfur were obtained. The original sample of grade 6 carbon black had a surface area of 108 square meters per gram. The extracted sample of black containing 0.4 per cent of combined sulfur had a surface area of 109 square meters per gram. These values are identical within experimental error.

While unaltered surface area need not necessarily be interpreted as evidence of complete lack of surface reactions, it is the authors' opinion that the extent of chemical combination at the carbon black surface is very small. This interpretation is in accord with the views expressed in a previous publication (*9*), where it was suggested that the chief role of carbon black in rubber reinforcement may rest on its ability to orient the chains of rubber molecules (*4*) and thus alter the extent and type of rubber-sulfur bonds normally formed in nonreinforced rubber stocks.

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Rapid Iodine Number Determinations

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OF THE numerous iodine number methods described in the literature for determining the unsaturation of fats and oils, the Wijs and Hanus methods are used most extensively (*4*), although the Rosenmund-Kuhnhenn method, especially in its micromodification, is used to a considerable extent in biological work. The Wijs reagent, official with the American Oil Chemists Society, gives theoretical values for pure nonconjugated unsaturated fatty acids, but it is sensitive to light and is not recommended for use when more than 30 days old. The Hanus reagent, on the other hand, usually gives results about 2 to 4 per cent lower than those obtained with the Wijs method, but it is stable and when protected from light will remain in a satisfactory condition for a year or longer. The Rosenmund-Kuhnhenn reagent is also stable but gives results appreciably low for all oils of iodine number greater than 100 (*2*). The reaction time for all three methods is usually 0.5 to 1 hour, depending upon the degree of unsaturation of the sample.

Some time ago, Hoffman and Green (*3*) suggested the addition of mercuric acetate to the Wijs reagent in order to cut the reaction time to 3 minutes without any change in the iodine number. This convenient rapid method has apparently escaped much attention. One of the present authors (*5*), however, has used this modified procedure on oils containing conjugated double bonds. Results obtained on tung oil are shown in Figure 1. Here the standard Wijs, rapid Wijs, and standard Rosenmund-Kuhnhenn methods are compared. Results obtained with the standard Hanus method are not shown, since they are known to be erratic when the reagent is employed in its normal concentration (0.2 *N*). [Theoretical iodine numbers on pure conjugated fatty acids have been reported by von Mikusch (*6*), using an approximately double strength Hanus reagent. However, normal strength Hanus reagent usually gives high and erratic results (*1*).]

Standard (slow) Wijs values are largely dependent upon the excess of reagent employed, the iodine number varying 43 units in an excess range of 25 to 272 per cent. Rapid Wijs values, however, vary only insignificantly in the reagent excess range of 30 to 225 per cent, permitting much greater latitude in sample weights. The 55 ± 3 per cent excess re-

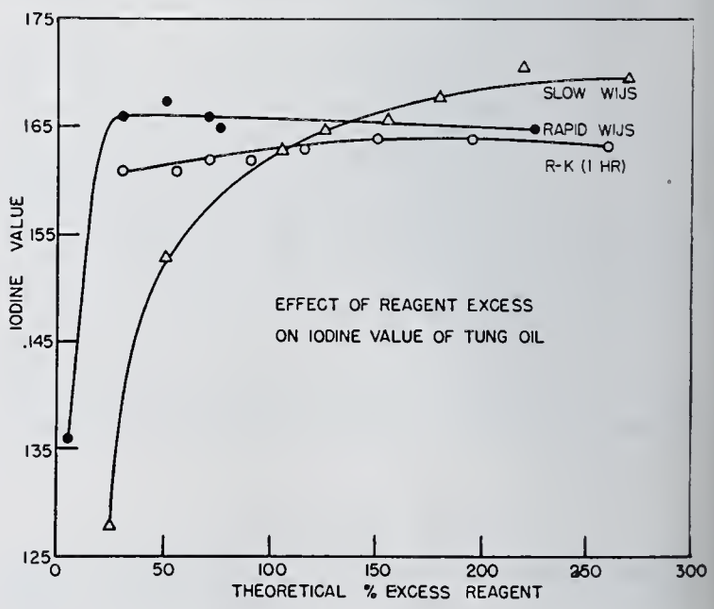

FIGURE 1

Table I. Comparison of Standard and Rapid Hanus Iodine Values on Unconjugated Fats

Fat	Iodine Value Standard Hanus	Iodine Value Rapid Hanus
Sardine oil	187.0	186.6
Linseed oil	173.4	174.0
Soybean oil	127.0	126.2
Corn oil	125.4	124.9
Cottonseed oil	109.2	109.5
Castor oil	84.0	90.7
Olive oil	84.6	85.0
Rendered pork fat	65.7	65.7

agent recommended for tung oil (*1*) in the standard Wijs method gives values located on the steep part of the curve and hence susceptible to maximum error. An excess range of 125 to 150 per cent would be a better choice if the standard Wijs method is used. The Rosenmund-Kuhnhenn method (1 hour) is second to the rapid Wijs in utility but the values obtained are slightly low, and if reaction time is shortened to 15, 30, or 45 minutes a further lowering of the iodine value is obtained, values tending to decrease gradually with decreasing reaction time below one hour.

Recently, the authors had need of an iodine number reagent suitable for intermittent use over fairly long periods of time. The stability of Hanus solution and the catalytic effect of mercuric acetate on iodine absorption of conjugated fats indicated that this combination be tried on nonconjugated materials. Their results are summarized in Table I.

With the single exception of castor oil, the standard and rapid Hanus iodine number methods check within the allowable experimental error (one unit). The anomalous result with castor oil is due to its content of ricinoleic acid, as indicated by experiments on a ricinoleic acid concentrate. With conjugated materials the rapid Hanus method is not satisfactory, since the iodine number varies with excess of reagent used in the determination. Representative results on a sample of tung oil are shown in Table II.

Experimental

Rapid Hanus Method. The iodine numbers were determined in the manner usually employed for the Hanus reagent (*4*) except that the 25 ml. of reagent were followed by 10 ml. of a 2.5 per cent solution of mercuric acetate in glacial acetic acid. The mixture was then shaken and titrated, after 3 to 5 minutes' standing. Rapid Hanus blanks were consistently found to be about 3.2 per cent lower than those of the standard Hanus method. Thus, the omission of mercuric acetate solution from the blank would increase the apparent iodine number of linseed oil by about two units.

Theoretical reagent excesses were calculated on the basis of an assumed iodine value of 170 for tung oil. For example, if the blank titration was 50.25 ml. and a 55 per cent excess of reagent was desired at the end of the reaction, then the titration of the sample should amount to $\frac{50.23}{1.55}$ or 32.41 ml. Assuming an iodine value of 170, then

$$170 = 32.41 \frac{(\text{grams of } I_2 \text{ per ml. of thiosulfate})}{\text{weight of sample}} \times 100$$

If 1 ml. of thiosulfate is equivalent to 0.013 gram of I_2,

$$\text{Weight of sample} = \frac{32.41\ (0.013)(100)}{170} = 0.248 \text{ gram}$$

Excesses calculated in this way are the only safe ones to use for conjugated fats, since otherwise the excess may be calculated on the basis of an incorrect experimentally obtained iodine value. This calculation is especially valuable for pure conjugated fatty acids since, in this case, the iodine value is known and need not be assumed.

Preparation of a Ricinoleic Acid Concentrate. The limited solubility of ricinoleic acid and its glycerides in petroleum ether was utilized in this preparation.

Castor oil (180 grams) was extracted five times with 100 ml. of petroleum ether (30° to 60°) at about −30° C. The residue (146.5 grams), freed from solvent, was saponified with 400 ml. of 20 per cent alcoholic potassium hydroxide, and the free fatty acids were liberated in the usual manner. Petroleum ether (200 ml.) was then added to dissolve the nonricinoleic acid unsaturated constituents, and the lower fatty acid layer was re-extracted with 100 ml. of petroleum ether. Following this, six cold extractions were made using petroleum ether at −65° C. (this warmed to about −10° or −20° C. after mixing with the fatty acids in a separatory funnel). The residue was then taken up in ethyl ether, washed free of sulfuric acid, the solution dried with anhydrous sodium sulfate, and the ether removed in vacuo.

The ricinoleic acid concentrate, melting point 6°, C. exhibited a rapid Hanus iodine number of 85.4 (theoretical, 85.1) and a standard Hanus value (0.5 hour) of 80.3, showing that ricinoleic acid is the substance responsible for the divergent results obtained on castor oil with the rapid and standard Hanus methods. Which of the two methods most nearly measures the true degree of unsaturation cannot be stated at this time, since it was not feasible to carry the purification procedure further. However, the product obtained by the method just outlined is likely to be contaminated with saturated and possibly some dihydroxy acids rather than with oleic or linoleic acids. Hence, the iodine number may be lower than expected on the basis of pure ricinoleic acid. Consequently, the low value obtained by the standard Hanus method may be due partly or entirely to contamination.

Table II. Effect of Reagent Excess on Rapid Hanus Iodine Values of Tung Oil

Sample Weight *Gram*	Excess Reagent Theoretical %	Excess Reagent Actual %	Iodine Value
0.0600	540	395	220.0
0.0600	540	392	220.3
0.1013	278	209	207.8
0.0996	284	213	207.3
0.1496	156	138	183.0
0.1492	157	138	183.7
0.2145	78.6	91.2	158.3
0.2140	78.8	93.4	157.1
0.2490	53.7	78.3	146.5
0.2485	54.1	78.2	146.8

Summary

The combination of mercuric acetate and Wijs solution provides a rapid iodine number method suitable for use on conjugated fats.

A rapid iodine number method involving the use of easily prepared and stable solutions comprises the combination of mercuric acetate with Hanus solution. This reagent gives values identical with those obtained in the standard Hanus method on nonconjugated fats, with the notable exception of castor oil, where the ricinoleic acid content is responsible for higher values obtained by the rapid method. On conjugated fats the method is unsatisfactory.

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Determining Glycerol in Crude Glycerin and in Soap Lyes

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A SIMPLE, rapid method for determining glycerol in crude glycerin and soap lyes has been needed for a long time. The international acetin method (*3*), though accurate, is lengthy and is applicable only to samples containing a maximum of 60 per cent water. The dichromate method (*1*) requires a preliminary purification and filtration, and its results tend to be high. The Bertram-Rutgers method (*2*), though simple and rapid, is lacking in accuracy. Distillation methods are usually lengthy and require special apparatus.

Experimental data (*5*) showed that the loss of glycerol from boiling aqueous glycerol solutions was negligible from a quantitative standpoint, and that the loss of glycerol from anhydrous 0.5-gram samples in small glass beakers at 100° C. was on the order of 2 mg. per hour. This latter fact indicated that a margin of safety could be expected for a quantitative method of removing water from aqueous glycerol solutions by evaporation. An accurate, quick method (*4*) had already been found for removing both water and glycerol from an aqueous glycerol sample. It was reasoned that, if water alone could be evaporated from an equal aliquot portion of the same sample, the percentage of glycerol could be calculated from the difference in weight of the two residues. The evaporation of water from an aqueous glycerol solution at 100° C. would be slow work at best. Therefore, to speed up the evaporation, many combinations of low-boiling solvents such as ethyl alcohol, ethyl ether, methyl alcohol, and benzene were tried. Methyl alcohol and ethyl ether were finally adopted as the most satisfactory.

Preparation of Sample

SOAP LYE CRUDE GLYCERIN AND SAPONIFICATION CRUDE GLYCERIN. Eight to 10 ± 0.001 grams are weighed into a tared 100-ml. measuring flask and about 50 ml. of water are added. The contents are well mixed, adjusted to the phenolphthalein end point with dilute hydrochloric acid or sodium hydroxide, made up to the 100-ml. mark with more water, and thoroughly mixed.

SOAP LYE. Thirty-five to 50 grams of lye are weighed into a tared 100-ml. measuring flask, the smaller sample for lyes of high salt content. The sample is adjusted to the phenolphthalein end point with hydrochloric acid or sodium hydroxide. One to 2 ml. of a 10 per cent aqueous solution of a wetting agent (Aerosol OT) are added, and the sample is made up to the 100-ml. mark with water and thoroughly mixed. The use of a wetting agent tends to reduce spattering.

Removal of Water from Aliquot Portions

Two 5-ml. aliquot portions of the sample are pipetted into tared 60-ml. Erlenmeyer weighing bottles (the bottles have a diameter of 50 mm. at the base, are 75 mm. high, and take a 24/12 ₹ stopper), and 5 ml. of methanol are added. The unstoppered bottles are placed in a natural convection drying oven maintained at 100° ± 2° C. The oven must have ample top and bottom vents for quick heat transfer, and its make and break contact points must be located below the samples for safety. Narrow strips of 0.6-cm. (0.25-inch) asbestos millboard placed on the metal shelf of the oven are effective in preventing spattering. The Sargent electric drying oven S-63995 has been found satisfactory.

When the residue in the bottles is reduced to a thin sirupy consistency by evaporation and the characteristic sweet odor obtained when glycerol is heated under these conditions is faintly perceptible, the bottles are removed from the oven. (The time varies between 65 and 80 minutes.) Five milliliters of ethyl ether are then added and swirled in the bottom of the bottles for 2 minutes. The bottles are put back into the oven and dried at 100° C. for about 25 minutes, until the acrid odor of ether is no longer perceptible and the odor associated with glycerol vapor is again noticeable. The bottles are then brought to room temperature in a desiccator over concentrated sulfuric acid, stoppered, and weighed to the fourth decimal place. The drying with the addition of 5 ml. of ether is repeated until the loss in weight is less than 0.002 gram. Usually, only one extra drying is necessary. The final weight, minus the tare, may be termed "residue at 100° C."

Removal of Both Water and Glycerol from Aliquot Portion

For this determination an infrared drying oven is used, either as described in a previous paper (*4*) or in the following manner: The top and bottom of an ordinary tin can, 15 cm. (6 inches) in diameter and 17.5 cm. (7 inches) high, are cleanly cut out, and four inverted V notches, about 2.5 cm. (1 inch) in height, are cut from the base. The can is mounted on its notched end upon a porcelain or asbestos surface. A 250-watt infrared reflector-drying lamp (General Electric R-40) is supported directly over the top opening of the tin can. A thermometer is inserted into one of the notches, so that its bulb rests directly beneath the center of the drying lamp.

Two 5-ml. aliquot portions of the sample are pipetted into tared, shallow, flat-bottomed evaporating dishes of about 70-mm. diameter. The tin can is set aside and the evaporating dishes are placed adjacent and on each side of the thermometer bulb. The tin can is replaced and current to the lamp is switched on. Preliminary evaporation is done with the lamp at a distance of about 20 cm. (8 inches) from the dishes. When the residue is almost dry, the lamp is raised slightly to prevent spattering of salt crystals. The temperature up to this point is disregarded. When the fumes of glycerol are scarcely to be seen coming off, the reading of the thermometer is brought to 160° C. by adjusting the height of the lamp. This temperature is maintained for 30 minutes, after which the evaporating dishes are removed, cooled to room temperature in a desiccator over concentrated sulfuric acid, and weighed rapidly to the fourth decimal place. This weight minus the tare may be termed "residue at 160° C." The over-all drying time rarely exceeds 90 minutes.

CALCULATION.

$$\frac{(\text{Residue at } 100^\circ \text{ C.} - \text{residue at } 160^\circ \text{ C.}) \times 100}{1/20 \text{ original sample}} = \%\text{ of glycerol}$$

Samples 1 and 2 are American Oil Chemists Society standard sample crude glycerin (February 1, 1929). The figure 83.33 per cent is the "true glycerol" content and represents the averaged findings of the laboratories of several soap companies. Samples 3 to 9, inclusive, are crude glycerins, both saponification and soap-lye, obtained from five different soap companies. The figures in the first column represent

TABLE I. EXPERIMENTAL RESULTS

Sample No.	Net Glycerol: Acetin method	Net Glycerol: Evaporation method	Difference
	%	%	%
1	83.33	83.66	0.33
2	83.33	83.51	0.18
3	85.04	85.20	0.16
4	79.62	79.80	0.18
5	78.23	78.39	0.16
6	88.20	88.45	0.25
7	88.07	88.26	0.19
8	89.56	89.85	0.29
9	90.37	90.38	0.01
	Glycerol expected	Glycerol found	Difference
	Gram	*Gram*	*Gram*
10	0.1655	0.1646	0.0009
11	0.3106	0.3111	0.0005

independent analyses by the acetin method. Samples 10 and 11 are "synthetic soap lyes", made from weighed amounts of the standard A. O. C. S. crude glycerin, salt, and water, to simulate the average soap lyes found in practice.

Summary

This method offers many advantages. The over-all time is about 4 hours, and the applied time is less than 1 hour. The technique is simple and the apparatus is readily available. The deviation from accepted or independent analyses of crudes is less than 5 parts per thousand, a fact that recommends it as a cost-accounting tool.

Although the method has not been perfected to the degree that the author would like, he feels that it is essentially useful as it stands and that refinements may be added when it is generally tried out.

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Determination of Tin in Babbitts, White Metal Alloys, and Bronze

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WHILE seeking a more satisfactory method for the determination of small amounts (<0.05 per cent) of tin in steel (*3*), the authors found that Stanreduce (Harshaw Scientific Co., Cleveland, Ohio) is an excellent reductant for the larger amounts of tin in bronze and white metal alloys.

This paper presents the noteworthy features of the improved methods developed for these alloys: simplicity, speed of reduction, and elimination of the use of rubber tubing and external gas supply. The three methods outlined should serve as a guide in developing a method adaptable to virtually any kind of alloy containing tin.

The authors make no claim to originality of method, the chemistry presented being essentially that first used by Low (*2*). They felt, however, that sufficient contradiction existed in the literature to merit a detailed study of the effects of the various manipulative procedures involved. The methods outlined are direct applications of data derived from this study.

Determination of Tin in Lead-Base Bearing Metal

The following procedure applies to lead-base bearing metal of the type of National Bureau of Standards sample No. 53, which contains: lead, 78.87 per cent; tin, 10.91; antimony, 10.09; bismuth, 0.06; copper, 0.05; iron, 0.05; and arsenic 0.02 (see Table I).

Place 2.0000 grams of a finely divided sample in a 300-ml. Erlenmeyer flask, add 5.00 grams of potassium sulfate and 15 ml. of concentrated sulfuric acid, and heat the mixture on a hot plate until the lead sulfate turns white. Cool the mixture, first in air and then for a moment with tap water, and dilute with 10 ml. of water followed by 150 ml. of 2 to 1 hydrochloric acid. Add 10 grams of Stanreduce and place a one-hole rubber stopper, through which passes a short capillary tube, lightly on the mouth of the flask. Place the flask on a hot plate at 150° C. and bring to a boil. Boil gently, maintaining the height of the foam at between 0.925 and 1.25 cm. (0.375 and 0.5 inch). Stopper the flask tightly, cover the capillary tube with a rubber policeman, and quickly remove the flask from the hot plate. Allow it to stand for about 2 minutes, during which time the hydrogen formed replaces the condensing steam, then place the flask in a water bath and cool to 30° C. or lower.

Into a 500-ml. Erlenmeyer flask pour 10 ml. of 1 to 2 hydrochloric acid and 5 ml. of starch solution, and add one 2.1-gram compressed sodium carbonate tablet. While the carbon dioxide is forming, fill a 100-ml. buret with standard potassium iodate solution, add a second bicarbonate tablet to the 500-ml. flask, withdraw the 300-ml. flask from the water bath, and remove the rubber policeman and then the rubber stopper. Now add 135 ml. of cold water to the 500-ml. flask and into it quickly decant the solution from the 300-ml. flask. Immediately wash the 300-ml. flask and Stanreduce with 1 to 2 hydrochloric acid and decant the washing into the 500-ml. flask. Add another sodium bicarbonate tablet as needed; a total of three or four tablets will suffice. Titrate at once to a blue end point. Without delay wash the Stanreduce with 10 ml. of 1 to 2 hydrochloric acid, decant into the 500-ml. flask, and again titrate. Repeat this latter procedure until a permanent end point is obtained.

Table I. Determination of Tin in Lead-Base Bearing Metal

(National Bureau of Standards sample 53, 10.91 per cent tin. 0.2182 gram of tin present)

Determination No.	Tin Found		Determination No.	Tin Found	
	Gram	%		*Gram*	%
1	0.2168	10.84	6	0.2196	10.98
2	0.2182	10.91	7	0.2186	10.93
3	0.2186	10.93	8	0.2192	10.96
4	0.2186	10.93	9	0.2192	10.96
5	0.2196	10.98	10	0.2182	10.91
			Mean	0.21866	10.93

Standardizing. Weigh out 0.2000-gram portions of pure tin, or use 2.0000-gram portions of National Bureau of Standards sample, and treat by the appropriate method.

Notes. Solution of the sample is obtained best at a temperature of 440° C. on a heater like the Gilmer. If a lead-base babbitt contains sufficient copper to interfere, tin should be determined as directed below for a tin-base babbitt. As a safety precaution, all flasks should be vacuum-tested and examined for flaws. The strong hydrochloric acid (2 to 1) is necessary to prevent the precipitation of antimony when large amounts are contained in the sample. The Stanreduce may be recovered for re-use by washing and drying. It should be sifted and the portion finer than 65-mesh discarded.

Determination of Tin in Tin-Base Bearing Metal

The following procedure applies to tin-base bearing metal of the type of National Bureau of Standards sample No. 54a, containing: lead, 0.21 per cent; tin, 88.61; antimony, 7.32; bismuth, 0.019; copper, 3.75; iron, 0.041; and arsenic, 0.031 (see Table II).

Place 0.5000 gram of the finely divided sample in a 300-ml. Erlenmeyer flask, and add 10 ml. of 1 to 1 nitric acid. When dissolved, evaporate the solution to dryness on a hot plate at 125° to 140° C. Bake for 15 minutes, digest the residue with 20 ml. of 1 to 1 nitric acid for 5 minutes, and then decant onto a 9-cm. No. 42 Whatman paper. By decantation, wash the metastannic acid in the flask three times with hot 2 per cent nitric acid, wash the contents of the filter paper three times with hot 2 per cent nitric acid, discard the filtrate, and return the filter paper to the flask.

Table II. Determination of Tin in Tin-Base Bearing Metal

(National Bureau of Standards sample 54a, 88.61 per cent tin. 0.4431 gram of tin present)

Determination No.	Tin Found		Determination No.	Tin Found	
	Gram	%		*Gram*	%
1	0.4437	88.74	6	0.4437	88.74
2	0.4437	88.74	7	0.4437	88.74
3	0.4437	88.74	8	0.4432	88.63
4	0.4432	88.63	9	0.4432	88.63
5	0.4426	88.52	10	0.4432	88.63
			Mean	0.4434	88.67

To the flask add 15 ml. of sulfuric-perchloric acid mixture (666 ml. of concentrated sulfuric acid, specific gravity 1.84, and 333 ml. of 72 per cent perchloric acid) and 2.0 grams of potassium sulfate, heat the mixture on a hot plate at 150° C. until all the carbon is oxidized, and then gradually increase the temperature to 400° C. to drive off the perchloric acid. Cool the solution, first in air and then for a moment with tap water, and dilute with 10 ml. of cold water, followed by 180 ml. of 1 to 2 hydrochloric acid. Starting with the addition of Stanreduce and increasing the time of reduction to 15 minutes, complete the determination as in the case of lead-base bearing metal, but omit the addition of the 135 ml. of cold water.

Notes. When the sample is dissolved in 1 to 1 nitric acid and evaporated to dryness, the product formed is a granular, sandlike precipitate of metastannic acid which filters out well. If concentrated nitric acid is used, the metastannic acid tends to become colloidal, and upon filtering may pass through the paper. In dissolving the metastannic acid, completion of solution is indicated when the flask clears. At this point, fumes of sulfuric acid only come from the mouth of the flask.

Determination of Tin in Bronze

The following procedure applies to bronze of the type of National Bureau of Standards sample No. 124, containing: copper, 83.77 per cent; zinc, 5.46; tin, 4.69; lead, 4.78; iron, 0.38; nickel, 0.45; and antimony, 0.23 (see Table III).

Place 1.0000 gram of the sample in a 300-ml. Erlenmeyer flask, and add 15 ml. of 1 to 1 nitric acid. Dissolve the sample on a hot plate at 150° C., add one half of a 9-cm. soft filter paper, and continue to heat and swirl until the paper is macerated. Dilute to 50 ml. with water and heat to boiling. Filter through a 9-cm. No. 42 Whatman paper containing a small amount of pulp, wash the flask once with hot 2 per cent nitric acid, and pour the washings into the filter paper.

Wash the filter paper five times with hot 2 per cent nitric acid, discard the filtrate, return the filter paper with its contents to the flask, and add 15 ml. of sulfuric-perchloric acid mixture. Then add 2.0 grams of potassium sulfate, heat the mixture on a hot plate at 150° C. until all the carbon is oxidized, gradually increase the temperature to 400° C., and continue heating until the perchloric acid is driven off. Cool the solution, first in air and then for a moment with tap water, and dilute with 10 ml. of cold water, followed by 180 ml. of 1 to 2 hydrochloric acid. Complete as in lead-base bearing metal, starting with the addition of Stanreduce, but omit the addition of the 135 ml. of cold water. The time of reduction can be reduced to 6 minutes.

Notes. Since metastannic acid is a colloidal precipitate, contamination is increased if the solution is evaporated to dryness, as some authors recommend (*1*). The process of evaporating to dryness consumes an excessive amount of time, and if the evaporation is hastened by overheating, cupric nitrate may be decomposed, with consequent contamination of the metastannic acid. If the analysis is carried out as directed with respect to acid concentration and filtration, the filtrate will be perfectly clear. Sufficient copper may remain in the metastannic acid to give a yellow tinge when hydrochloric acid is added, but the authors have found this insufficient to do any harm.

Table III. Determination of Tin in Bronze

(National Bureau of Standards sample 124, 4.69 per cent tin. 0.0469 gram of tin present)

Determination No.	Tin Found		Determination No.	Tin Found	
	Gram	%		*Gram*	%
1	0.0467	4.67	6	0.0462	4.62
2	0.0467	4.67	7	0.0462	4.62
3	0.0462	4.62	8	0.0462	4.62
4	0.0462	4.62	9	0.0462	4.62
5	0.0462	4.62	10	0.0462	4.62
			Mean	0.0463	4.63

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Sugar Analysis by Alkaline Ferricyanide Method

Determination of Ferrocyanide by Iodometric and Other Procedures

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One of the problems of special interest when reducing sugars are to be estimated by oxidation with alkaline ferricyanide solutions is the selection of the method for determination of the amount of reagent which has been consumed. The reduction product, ferrocyanide, is frequently estimated volumetrically by titration with one of the stronger oxidizing agents.

Burriel and Sierra (*2*) titrated ferrocyanide satisfactorily with potassium dichromate in 0.3 *N* hydrochloric acid solution, using benzidine acetate as indicator. Muller and Diefenthaler (*5*) employed permanganate in a solution acidified with sulfuric acid. Furman and Evans (*3*) used ceric sulfate in hydrochloric acid solution with *o*-phenanthroline as indicator. However, as they have shown, cerous compounds may be oxidized to ceric by ferricyanide in 30 per cent potassium carbonate solution. Where only ferrocyanide is to be considered, the use of some one of these stronger oxidizing agents generally is to be preferred. The presence of the oxidation products of the sugars along with the ferrocyanide raises the question as to whether these substances may not also have reducing action upon the reagent used to titrate the ferrocyanide.

Strepkov (*8*) reports that oxidizing of dextrose and levulose with Ost's solution, removing the cuprous oxide, then adding a definite amount of ferrocyanide to the residual solution containing the oxidation products of the sugars, and titrating the mixture with potassium dichromate gave results which were in good agreement with the blanks containing only the ferrocyanide. On the other hand, parallel experiments in which the sugars were oxidized by a Fehling's alkaline-tartrate mixture showed a greater consumption of dichromate than was given by the blanks. Use of a weaker oxidizing reagent to estimate ferrocyanide would appear to be advantageous in many instances where other reducing products than ferrocyanide are present.

Of the weaker oxidizing reagents, iodine has been employed for the titration of ferrocyanides. Considerable controversy is apparent as to the conditions under which the reaction should be carried out. In neutral or weakly acid solution the reaction goes to the right, while in solutions more strongly acidified it is reversed:

$$2K_4Fe(CN)_6 + I_2 \underset{}{\overset{H^+}{\rightleftharpoons}} 2K_3Fe(CN)_6 + 2KI$$

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Table I. Effect of Buffer and Complexing Agents in Neutral and Acetic Acid Solutions

[Oxidation of $K_4Fe(CN)_6$ with 0.02 *N* iodine]

Reagents Used	$K_4Fe(CN)_6$ Taken	$K_4Fe(CN)_6$ Found	Error
	Milliequivalents		%
(*a*) $NaHCO_3$ 10 ml. of $K_4Fe(CN)_6$, 20 ml. of I_2, 50 ml. of water, and enough $NaHCO_3$ to make the solution 1% in this reagent	0.2068	0.1165	43.7
Same as above except acidified with 20 ml. of 20% acetic acid before titrating	0.2068	0.1990	3.8
(*b*) No complexing reagent used 10 ml. of $K_4Fe(CN)_6$ and 50 ml. of water heated to 40° C. before 20 ml. of I_2 solution added	0.2355	0.1889	19.8
Same as above except acidified as in (*a*)	0.2328	0.2055	11.7
(*c*) Rochelle salt 10 ml. of $K_4Fe(CN)_6$, 20 ml. of I_2, 50 ml. of water, and 5 grams of Rochelle salt	0.2355	0.1726	26.7
Same as above except acidified as in (*a*)	0.2068	0.2018	2.4
(*d*) $Na_2HPO_4.12H_2O$ 10 ml. of $K_4Fe(CN)_6$, 20 ml. of I_2, 25 ml. of water, and 25 ml. of 10% $Na_2HPO_4.12H_2O$	0.2343	0.1650	29.6
Same as above except acidified as in (*a*)	0.2179	0.2178	0.0
(*e*) K_2F_2 10 ml. of $K_4Fe(CN)_6$, 20 ml. of I_2, 30 ml. of water, and 20 ml. of 3% K_2F_2	0.2284	0.1654	27.6
Same as above except acidified as in (*a*)	0.2284	0.2274	0.4

Rupp and Schiedt (*7*) have reported good results by the addition of an excess of the standard iodine to a bicarbonate-buffered solution of the ferrocyanide, then titration of the excess of iodine with thiosulfate. Muller and Diefenthaler (*5*) object to the use of the sodium bicarbonate. These authors believe the results obtained by the previous workers were apparently satisfactory because of a compensation of errors. One factor, an oxidation of sodium thiosulfate to sodium sulfate by hypoiodite, tends to require too little thiosulfate, while the conversion of some iodine to iodate in the slightly alkaline solution, not requiring thiosulfate, tends to offset the first effect. The degree of alkalinity, amount of excess iodine, and period of reaction affect the results. They report fairly satisfactory data if the excess iodine in the bicarbonate-buffered solution is titrated with an arsenious reagent, provided the solution does not stand so long that iodate formation has taken place. In a second article Rupp (*6*) recommends the use of sodium potassium tartrate instead of bicarbonate. Muller and Diefenthaler were unsuccessful with this procedure also. More recently Kolthoff (*4*) has reviewed the general problem, emphasizing in addition to the factors already mentioned, the effect of complexing the Fe^{+++} to shift the equilibrium in the desired direction and titration of the excess iodine in acid solution. Furthermore, the danger of error as a result of atmospheric oxidation is pointed out. Kolthoff proposes a method in which the ferrous solution is treated with potassium bromate and later potassium iodide in phosphoric acid solution containing a trace of molybdate as a catalyst. Very good results are reported.

Kolthoff states that no benefit was observed from the presence of sodium potassium tartrate in neutral solution. In fact, better results were obtained when it was left out but the results were still 2 per cent too low. By first heating the solution to 40° C., then adding iodine in excess, and back-titrating with thiosulfate, he reported good results.

In view of the somewhat uncertain state of affairs with regard to the iodometric determination of ferrocyanide, further work in this connection was undertaken with particular reference to the application of the determination in processes of sugar analysis.

Experimental

All the chemicals used were of analytical grade and the reactions were carried out in glass-stoppered flasks with all the usual precautions observed in iodometric work.

Preliminary experiments with the bicarbonate buffer method of Rupp and Schiedt (*7*) confirmed the observations of later workers as to low results. In all cases more nearly correct results were obtained by slightly acidifying with acetic acid just before titration of the excess iodine with thiosulfate, but the results were still low because of the slight reversibility of the reaction. The elevated temperature method of Kolthoff in the authors' hands was also unsatisfactory. Use of tartrate in the reaction mixture as proposed by Rupp (*6*) gave low results in the nearly neutral solution, but if acidified before the excess iodine was titrated the results were only slightly low. A precipitate of potassium acid tartrate caused the solution to become cloudy and interfered somewhat with the indicator reaction.

The improvement in results noted when the tartrate was present may be assigned to the complexing of the ferric ion and shift of the equilibrium in the desired direction. The effect is similarly emphasized by the satisfactory results reported by Kolthoff with his procedure using the bromate-iodide mixture, acidifying with phosphoric acid, and then titrating the excess iodine with thiosulfate. Tests with phosphate and fluoride as complexing agents were also included in the preliminary experiments and sample results are shown in Table I.

Although the concentration of phosphate or fluoride and that of the acetic acid of acidification did not appear to be critical, the effect of the excess of iodine solution added for the oxidation and the time which it stood in contact with the ferrocyanide solution did seem to be important.

These factors were studied in a series of tests in which 10.00 ml. of 0.02179 *N* potassium ferrocyanide solution, 25 ml. of 20 per cent acetic acid, and 25 ml. of 10 per cent disodium hydrogen phosphate dodecahydrate were used with varying amounts of the 0.02 *N* standard iodine solution. The solutions were permitted to stand in a 150-ml. glass-stoppered flask for varying lengths of time and then titrated to the starch end point with a standard thiosulfate solution. From the results of Table II it is apparent that when there is a 65 to 70 per cent excess of the iodine reagent the oxidation of the ferrocyanide is accomplished within 15 minutes. The use of elevated temperature is not necessary, since the reaction is complete at room temperature in this relatively short time.

Table II. Effect of Time, Temperature, and Excess of 0.02 *N* Iodine upon Oxidation of Ferrocyanide

[10 ml. of 0.02179 *N* $K_4Fe(CN)_6$, 25 ml. of 10% $Na_2HPO_4.12H_2O$, and 25 ml. of 20% acetic acid used in each determination]

Experimental Conditions Used: Temperature	Time	Iodine	Iodine in Excess of Amount Consumed	$K_4Fe(CN)_6$ Taken	$K_4Fe(CN)_6$ Found	Error
° *C.*	*Min.*	*Ml.*	%	*Milliequivalents*		%
50	15	25	115	0.2179	0.2163	−0.7
26	15	10	10	0.2179	0.2163	−0.7
26	15	15	65	0.2179	0.2178	0.0
26	15	20	119	0.2179	0.2185	0.3
26	15	25	174	0.2179	0.2178	0.0
26	15 sec.	15	72	0.2179	0.2076	−4.7
26	5	15	65	0.2179	0.2174	−0.2
26	15	15	65	0.2179	0.2178	0.0
26	11 hours	25	174	0.2179	0.2178	0.0

When the same procedure was applied to 0.1 *N* ferrocyanide and iodine solutions, the results were consistently 1 per cent too low and the end point was somewhat fugitive. However, by using a 300-ml. flask and adding 100 ml. of water to the reaction mixture before the thiosulfate titration, theoretical values and a stable end point were obtained. This fact is demonstrated in the following experiments:

Large crystals of potassium ferrocyanide trihydrate were split apart and portions of the center section which showed no evidence of dehydration were used for a series of determinations. The weighed portion of ferrocyanide (not in excess of 0.6 gram) in a 300-ml. glass-stoppered flask was dissolved in about 35 ml. of water and then 25 ml. of 20 per cent acetic acid, 25 ml. of 10 per cent disodium phosphate solution, and 25 ml. of standard 0.1 *N* iodine solution were added. After the flask had stood in a dark place for 15 to 20 minutes, 100 ml. of water were added and the excess iodine was titrated with thiosulfate to a starch end point.

In a second series of experiments 3 per cent potassium fluoride was substituted for the phosphate.

The results are given in Table III.

Table III. Determination of Potassium Ferrocyanide

$K_4Fe(CN)_6.3H_2O$ Taken	$K_4Fe(CN)_6.3H_2O$ Found	Error
Gram	*Gram*	%
0.1 *N* iodine as oxidizing agent in slightly acid solution, Na_2HPO_4 as complexing agent		
0.4631	0.4636	0.0
0.4760	0.4788	0.6
0.4650	0.4638	−0.3
0.4710	0.4717	0.1
0.4639	0.4627	−0.3
0.4100	0.4113	0.3
0.3290	0.3293	0.1
0.3361	0.3360	0.0
0.4798	0.4798	0.0
0.4327	0.4339	0.3
0.4740	0.4751	0.2
0.3492	0.3482	−0.3
0.2608	0.2621	0.5
0.3832	0.3831	0.0
0.1 *N* iodine as oxidizing agent in slightly acid solution, K_2F_2 as complexing agent		
0.3854	0.3858	0.1
0.3942	0.3934	−0.2
0.4126	0.4124	0.0
$Ce(SO_4)_2$ as oxidizing agent		
0.8809	0.8814	0.1
0.8977	0.8964	−0.1

Table IV. Comparison of Methods for Determining Amount of Ferricyanide Reduced after Oxidation of Sugars

[5 mg. of levulose or dextrose heated at 50° C. with 25 ml. of a reagent containing 4 grams of $K_3Fe(CN)_6$, 80 grams of $Na_2HPO_4.12H_2O$, and 150 grams of Na_2CO_3 per liter]

Heating	$K_4Fe(CN)_6$ by Iodometric Method		$K_4Fe(CN)_6$ by $Ce(SO_4)_2$ Method		Excess $K_3Fe(CN)_6$ by KI Method	
	Dextrose	Levulose	Dextrose	Levulose	Dextrose	Levulose
Min.	*Milliequivalent of apparent ferrocyanide formed*					
15	0.0034	0.0826	0.0053	0.0810	0.0051	0.0810
30	0.0106	0.1305	0.0115	0.1305	0.0110	0.1305
45	0.0161	0.1539	0.0178	0.1550	0.0167	0.1552
60	0.0224	0.1645	0.0233	0.1663	0.0226	0.1667
75	0.0280	0.1727	0.0285	0.1721	0.0290	0.1740

Variations of the experimental value from the quantity taken are small in all cases and deviate slightly in both directions from the theoretical. Better consistency can, of course, be obtained by using aliquots from a standard solution where slight errors in weighing and variation in composition of sample are eliminated. It is apparent that the potential of the ferric complex is lowered to the point at which iodine is not formed from iodide at room temperature even in acetic acid solution.

The hydrofluoric acid liberated by the acetic acid when potassium fluoride was used as a complexing agent was without effect upon Pyrex flasks over a period of several weeks, but a soft-glass stirring rod and the bulb of a thermometer were noticeably etched during this time. There seems to be no preference as to the use of the disodium phosphate or the potassium fluoride with respect to complexing ability. The solutions were about 0.03 *M* with respect to each of these salts.

The low results at first encountered when 0.1 *N* reagents were used were never observed when 0.02 *N* reagents were employed nor were end-point difficulties encountered in the latter case. That the trouble was due in the first case to a concentration effect is demonstrated by the fact that the addition of water made possible satisfactory results.

Recommended Procedure

The ferrocyanide content of an unknown sample is determined in the following manner:

The ferrocyanide or material containing the ferrocyanide must be put into solution if it is a solid. If it is necessary to use acid for this purpose, the solution must be neutralized before the analysis is started. The volume of the ferrocyanide solution should be from 25 to 50 ml. and should contain not over 1.5 milliequivalents of ferrocyanide (0.6 gram of $K_4Fe(CN)_6.3H_2O$). To this amount of the approximately neutral ferrocyanide solution in a 300-ml. glass-stoppered flask are added 25 ml. of 10 per cent disodium phosphate dodecahydrate (or 25 ml. of 3 per cent potassium fluoride), 25 ml. of 20 per cent acetic acid, and 25 ml. of 0.1 *N* standard iodine solution. The flask is set in the dark for 15 to 20 minutes and then 100 ml. of water are added before the excess iodine is titrated to a starch end point with thiosulfate.

The strength of the iodine solution can be varied to cover different concentrations of ferrocyanide, as long as the volume of solution is large enough to keep the ferricyanide concentration in the reaction mixture below 0.01 *M* and a 65 to 100 per cent excess of iodine is used.

Determination of the Extent of Reduction of Alkaline Ferricyanide after Use of the Reagent for Oxidation of Dextrose and Levulose

Having confirmed the practicability of the iodometric estimation of ferrocyanide, a series of experiments was planned to determine the nature of the agreement of the results when the extent of reduction of alkaline ferricyanide by sugars was estimated by several methods. Three methods were employed: the iodometric procedure described above; the ceric sulfate titration of ferrocyanide as used by Becker and Englis (*1*); and, indirectly, determination of the excess ferricyanide.

The last-mentioned was carried out according to Kolthoff (*4*) by making the solution 3 *N* with hydrochloric acid, adding a 1 *N* potassium iodide solution, and titrating the iodine with thiosulfate after the mixture had stood for about 5 minutes in a glass-stoppered flask. The conditions for the sugar oxidation were those previously described for the selective oxidation of levulose (*1*). Since under these conditions dextrose is only slightly oxidized and the products of oxidation left might be expected to respond somewhat differently from those after oxidation of levulose, experiments were carried out with each of the sugars. The results are given in Table IV.

On the whole, the agreement among the methods is very good and is of the order of precision to be expected in sugar analysis. Slight variations may be assigned to probable variation in the sugar oxidation itself rather than to the estimation of the ferrocyanide. If there is any slight further oxidation of the by-products of the action of the ferricyanide on the sugars by the ceric sulfate, it is within experimental error.

Summary

The oxidation of ferrocyanide with iodine may be accomplished by carrying out the reaction in slightly acid solution in the presence of a reagent such as phosphate or fluoride to complex the iron and prevent the reverse reaction.

The oxidation may be carried out at room temperature with a 60 to 75 per cent excess of iodine and a time interval of about 15 minutes The volume of the reaction mixture must be such that the ferricyanide concentration is below 0.01 *M*. The excess iodine is titrated with standard thiosulfate.

A comparison of results obtained when the reduction of alkaline ferricyanide by dextrose and levulose was made by direct oxidation of the ferrocyanide with iodine, indirect estimation by determination of ferricyanide iodometrically, and direct oxidation of ferrocyanide with ceric sulfate showed good agreement and indicates that the by-products of the primary oxidation of the sugars have a negligible effect upon any of the methods used to estimate the ferricyanide consumed.

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Determination of Chloride in the Presence of Hydrosulfide or Sulfide Ion

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A method is proposed for the determination of chloride in the presence of hydrosulfide (or sulfide) ion. The procedure involves the precipitation of the hydrosulfide as cupric sulfide, removal of the latter by quantitative filtration, and determination of the chloride gravimetrically as silver chloride. Results are reported of analyses of several samples ranging from 94 to 99 per cent sodium chloride, the remainder being sodium hydrosulfide. The average error is −0.08 per cent; the greatest error is −0.19 per cent.

IN THE course of an attempted synthesis of a heretofore unknown alkyl mercaptan, it was naturally desired to calculate the yield of finished product. Two paths were open: to calculate the yield from the weight of the purified sulfur compound obtained, or to base calculations on the amount of sodium chloride recovered as a by-product. As a result of using the former and customary procedure, extraordinarily high yields of mercaptan were obtained. It was decided, therefore, to check these yields by employing the alternative method.

Surprisingly, there is very little information in the literature bearing directly on the determination of chloride in the presence of sulfide, hydrosulfide, or both.

Wyler (*4*) determined the concentration of chlorides, including sodium chloride, in mixtures containing the sodium salt of the sulfide, hydrosulfide, thiosulfate, sulfate, carbonate, and chloride. His method consists principally in the oxidation of the sulfide and hydrosulfide to sulfate by means of chromic acid, precipitation of the sulfate together with the chloride with silver ion, and subsequent isolation of the precipitate. The precipitate is then dissolved in aqueous ammonia and the silver chloride is reprecipitated by means of nitric acid. The silver sulfate remains in solution.

Topsöe (*2*) developed a method for the estimation of chlorides, bromides, or iodides in the presence of hydrogen sulfide. His procedure consists essentially in the oxidation of hydrogen sulfide to sulfate by means of acidic potassium permanganate, conversion of the liberated halogen into the corresponding hydrogen compound by sulfurous acid, reduction of the excess permanganate by the same reagent, and determination of the haloids in the usual way.

Another method (*1*) suggests acidification with nitric acid, with subsequent expulsion of the hydrogen sulfide generated by boiling. The halide is then determined by means of the Volhard method (*3*).

Wyler's method (*4*) was tried several times in this laboratory and was found to give satisfactory results. However, the method is time-consuming and was therefore objectionable. Khadanov's method (*1*) gave excellent results with synthetic mixtures of sodium chloride and sodium hydrosulfide, but failed to yield precise values with the impure sodium chloride obtained from the synthesis of the mercaptan. The treatment was the same in each case. It is possible, however, that traces of mercaptan, which were undoubtedly present in the impure sodium chloride, were responsible for the discrepancies. Check determinations varied by as much as 0.3 per cent.

It was desirable for the authors' purpose to develop a shorter method, in view of the fact that many analyses were contemplated. The method described below involves precipitation of the hydrosulfide or sulfide as cupric sulfide. This is separated from the soluble chlorides by filtration and the latter, in turn, are precipitated with silver nitrate. The silver chloride may then be determined gravimetrically.

Known mixtures of sodium chloride–sodium hydrosulfide ranging from 94 to 99 per cent of the former were prepared. This range was selected because it corresponded to the expected "purity" of the sodium chloride precipitated from the authors' experiments.

In several preliminary experiments, it was found that lead nitrate may be substituted for the cupric salt, if certain precautions are observed. The lead sulfide must be filtered from a hot solution (60° to 70° C.), otherwise lead chloride, which is fairly insoluble in the cold, will also be removed. It is advisable to precipitate the silver chloride from a hot solution to prevent occlusion. Oddly, no occlusion effects were observed.

If desired, the silver chloride may be determined volumetrically in the usual way (*3*) by adding an excess of standard silver nitrate, filtering the silver chloride, and titrating the excess silver ion with standard ammonium thiocyanate solution, using ferric ammonium sulfate as the indicator.

Reagents

Chemically pure sodium hydrosulfide dihydrate, reagent-quality sodium chloride, a 5 per cent aqueous solution of C. P. cupric nitrate trihydrate, a 5 per cent aqueous solution of C. P. nitric acid, and a 5 per cent aqueous solution of reagent-quality silver nitrate.

Experimental Procedure

99 PER CENT SODIUM CHLORIDE–1 PER CENT SODIUM HYDROSULFIDE MIXTURE. Samples of sodium chloride and sodium hydrosulfide dihydrate in the ratio of 19.8083 to 0.2018 gram were weighed directly into a 500-ml. volumetric flask. Distilled water was added to the mark and solution effected. A 100-ml. aliquot was taken and acidified with 5 per cent nitric acid to a pH of 4 on Universal indicator paper. Next 10 ml. of 5 per cent cupric nitrate solution were added immediately with stirring to precipitate cupric sulfide. The mixture was then allowed to stand for 15 minutes and filtered quantitatively at the end of this time. The filtrate was transferred, again quantitatively, to a 500-ml. volumetric flask, made up to the mark with distilled water, and the solution was mixed thoroughly. A 25-ml. aliquot was diluted to 100 ml., 5 ml. of 5 per cent nitric acid were added, and the chloride ion was precipitated as silver chloride upon the addition of 15 ml.

TABLE I. DETERMINATION OF CHLORIDE

Sample	Composition		Amount of AgCl Calculated	Amount of AgCl Experimental	Error
	%		*Gram*	*Gram*	%
1	99	NaCl	0.4859	0.4859	0.00
	1	$NaSH.2H_2O$		0.4855	−0.08
				0.4855	−0.08
2	97	NaCl	0.4759	0.4750	−0.19
	3	$NaSH.2H_2O$		0.4754	−0.11
				0.4758	−0.02
3	94	NaCl	0.4611	0.4609	−0.04
	6	$NaSH.2H_2O$		0.4605	−0.13
				0.4607	−0.08
				Av.	−0.08

of 5 per cent silver nitrate. The mixture was allowed to stand for several hours and filtered. The precipitate was dried to constant weight at 110° C.:

$$\text{Weight of NaCl} \times \frac{100}{500} \times \frac{25}{500} \times \frac{\text{M. W. of AgCl}}{\text{M. W. of NaCl}} =$$

$$\text{weight of NaCl} \times 0.02453 = \text{weight of AgCl (calculated)}$$

$$19.8083 \times 0.02453 = 0.4859 \text{ gram of AgCl (calculated)}$$

97 Per Cent Sodium Chloride–3 Per Cent Sodium Hydrosulfide Mixture. The procedure was the same as that outlined above, except that the mixture was obtained by weighing out 19.4000 and 0.6000 gram of sodium chloride and sodium hydrosulfide, respectively:

$$19.4000 \times 0.02453 = 0.4759 \text{ gram of AgCl (calculated)}$$

94 Per Cent Sodium Chloride–6 Per Cent Sodium Hydrosulfide Mixture. The procedure was again the same as that outlined above, with the exception that the original mixture was composed of 18.8000 grams of sodium chloride and 1.2000 grams of sodium hydrosulfide:

$$18.8000 \times 0.02453 = 0.4611 \text{ gram of AgCl (calculated)}$$

The results are given in Table I. The average error of the analyses on three samples is −0.08 per cent; the greatest individual error is −0.19 per cent. The reproducibility and accuracy, as seen from these results, are excellent.

Work is now in progress on the quantitative determination of the sulfide content of these mixtures. These results will be published at a later date.

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Determination of Carotene in Vegetable Oils without Saponification

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The increasing research on carotene and its use in vegetable oil solutions has called attention to the desirability of a simple, accurate method for determining the carotene content of such solutions. The methods at present available are either too long and tedious or are subject to errors which may in some cases be large.

The more rapid methods depend upon the determination of total color by simple dilution of the oil sample with a suitable solvent and measurement of the percentage of light transmission by the solution. Data presented in this paper show that this method may be in considerable error because of the presence of colored substances that form during storage of the carotene solutions and which are included with the carotene in the analysis.

The longer methods, which are the more accurate, all require that the sample be saponified. The Guilbert method (*4*) as modified by Peterson *et al.* (*10*) and by Peterson (*9*) may be regarded as representative of the saponification methods. Following the saponification approximately 12 phasic partitions are made in separatory funnels. Caution is necessary to prevent the formation of rather stable emulsions in this process. The noncarotene chromogens are removed by partition between nonmiscible solvents or by use of chromatographic adsorption columns (*11*).

Selection of Adsorbent

A rapid chromatographic method without the time-consuming saponification and phasic separations is desirable.

Vegetable oil solutions of carotene and noncarotene chromogens were dissolved in petroleum ether and analyzed for carotene, using the adsorbents suggested by Moore (*7*), Kernohan (*6*), and Wall and Kelley (*14*) for the analysis of plant extracts. It was found that these adsorbents were not directly applicable to oil solutions, as the presence of the oil interfered with the adsorption of the noncarotene chromogens by the dibasic calcium phosphate, soda ash, and magnesium oxide–Celite columns. In the method of Fraps, Kemmerer, and Greenberg (*3*) special techniques are required for preparing the adsorbents, and the degree of activation may vary on storage, so that their activity must be redetermined each time they are used. For these reasons no attempt was made to apply the method to analysis of solutions containing oil.

Seaber analyzed oil samples for carotene by saponification, removal of alkali and alcohol, transference of carotene to petroleum ether and adsorption of the carotenoids on aluminum oxide standardized according to Brockman, and eluting the carotene with 3 per cent acetone in petroleum ether.

Thaler (*13*) prepared chromatograms of various fats and oils on Tswett columns of aluminum oxide in order to detect the presence or absence of artificial colors in the oils, but made no attempt at a quantitative separation of the carotene from the other associated pigments.

This work suggested that aluminum oxide (<80-mesh aluminum oxide activated especially for chromatographic analysis, manufactured by the Aluminum Ore Company, East St. Louis, Ill.) might be used for separating carotene directly from other chromogens present in oil, thus avoiding saponification. With solutions of purified crystalline carotene, aluminum oxide columns caused no detectable destruction of carotene. Various amounts of acetone in petroleum ether were tested as eluents. Two per cent acetone in petroleum ether was found to remove carotene quantitatively from the aluminum oxide column and permit its separation from the colored oxidation products of carotene, xanthophylls, and chlorophylls. Higher concentrations of acetone did not permit as sharp a separation of carotene from the colored impurities, as they followed the carotene through the column too closely.

Procedure

Tswett tubes having an inside diameter of 11 mm. are packed with 12 grams of the aluminum oxide. Columns of uniform height of about 10 cm. are obtained by applying gentle suction and tamping lightly with a heavy glass rod spread and flattened on one end. The column is covered with about 1 gram of anhydrous sodium sulfate. A 10-ml. aliquot of a petroleum ether (boiling point 30° to 60° C.) solution, containing 0.1 gram of the oil per ml. of solution, is run onto the column. After all the solution has passed into the aluminum oxide the column is developed and the carotene is simultaneously eluted with the required amount of petroleum ether containing 2 per cent acetone (no pressure or vacuum is required to hasten the percolation).

The volume of eluent needed must be determined for each batch of aluminum oxide. As the commercial preparation of aluminum oxide may vary somewhat in activity from one lot to

Table I. Separation of Carotene from Oxidation Products of Carotene and from Xanthophyll

(Micrograms of carotene in 10-ml. aliquot)

Sample	Description of Sample	Apparent Carotene by Dilution: No oil	Apparent Carotene by Dilution: Oil added[a]	Carotene by Al_2O_3 Column: No oil	Carotene by Al_2O_3 Column: Oil added[a]	Carotene by CaHPO Column: No oil
1	10 ml. of a solution of pure carotene	140	142	138	140	138
2	10 ml. of a petroleum ether solution of carotene containing a small amount of oxidized carotene	134	139	127	127	128
3	10 ml. of a petroleum ether solution of carotene containing a large amount of oxidized carotene	173	175	100	100	99
4	1 ml. of a petroleum ether solution of xanthophyll (X) diluted with petroleum ether to 10 ml.	26	...	<1	...	<1
5	1 ml. of a petroleum ether solution of carotene and oxidized carotene (C) diluted to 10 ml.	61	...	53	...	53
6	2 ml. of solution (C) + 7 ml. of solution (X) diluted to 10 ml.	301	308	...	107	107
7	4 ml. of solution (C) + 2 ml. of solution (X) diluted to 10 ml.	270	275	...	211	212

[a] 1 gram of corn oil was added to these samples before dilution to 10 ml.

another, a preliminary determination of the volume of eluent is required. The required calibration may be readily made as follows: A 10-ml. aliquot of a petroleum ether solution of crystalline carotene, containing 0.1 gram of oil per ml. of solution, is run onto the column. Several columns are prepared in this manner. Columns are then washed with various amounts of 2 per cent acetone in petroleum ether to determine the minimum amount of eluent necessary to remove all the carotene. This volume is then used in the analyses for eluting the carotene and leaving the other colored impurities on the column. The columns prepared from the first three 2.27-kg. (5-pound) lots of aluminum oxide required 45 ml. of eluent to remove the carotene, while the columns prepared from the fourth 2.27-kg. (5-pound) lot required 65 ml. of eluent. The aluminum oxide must be protected against the adsorption of moisture to keep its activity constant.

The carotene eluate is caught in a 100-ml. volumetric flask and made to volume, and the carotene concentration is determined colorimetrically in an Evelyn photoelectric colorimeter with a 440-mμ filter. This measures beta-carotene together with any alpha- or neo-beta-carotene that may be present. Both alpha- and neo-beta-carotene are more readily eluted from the aluminum oxide column than beta-carotene. Methods are available for the individual estimation of these components by spectrophotometric technique (*12*, *15*).

The method can also be used to separate carotene from other pigments in a petroleum ether solution that is free of oil. More eluent is needed to remove the carotene from the column when no oil is present. The volume of eluent required may be readily determined as described above by use of a petroleum ether solution of carotene containing no oil. The volume of eluent required for columns prepared from various lots of aluminum oxide ranged from 75 to 90 ml.

Comparison with Simple Dilution and Moore Method

The proposed method was tested and evaluated in the following manner:

Petroleum ether solutions of pure carotene and carotene containing some oxidized carotene were analyzed by three methods: (1) by simply diluting with petroleum ether to a suitable volume, (2) by the proposed method, and (3) by using a column of dibasic calcium phosphate as recommended by Moore (*7*).

Moore (*8*) has shown that the dibasic calcium phosphates obtained commercially vary considerably in activity and that potassium hydroxide will activate the commercial product. These results were confirmed in this laboratory and extended to show that commercial dibasic calcium phosphate from two manufacturers could be activated to the same degree by the following process: 500 grams of commercial dibasic calcium phosphate and 40 grams of potassium hydroxide are added to 5 liters of water in a 6-liter boiling flask. The flask is immersed in boiling water for 45 minutes, and the contents are stirred every 5 minutes. The calcium phosphate is then filtered on a Büchner funnel. Suction is applied until the mass is sufficiently dried so that the surface just begins to crack. The mass is then washed five times with 100-ml. portions of distilled water. The phosphate is dried for 24 hours at 105° C. Each batch so activated is tested with a pure solution of carotene in petroleum ether, to which a known amount of oxidized carotene is added. The proper activation is reached if the column retains all the impurity without retaining or destroying any carotene.

The same solutions of carotene, with the addition of 0.1 gram of vegetable oil per ml., were analyzed by the first and second methods. The data (Table I, sample 1) showed that neither the aluminum oxide nor the calcium phosphate column caused any destruction of carotene and that all the carotene could be recovered. Samples 2 and 3 were solutions of purified carotene in petroleum ether that had developed colored oxidation products of carotene during storage. Therefore, analysis by simple dilution (first method) gave higher value than the other two methods which removed these noncarotene chromogens. Analyses of oil-free solutions of carotene containing some oxidized carotene, by the proposed method and by the dibasic calcium phosphate column, gave the same values. Analyses of oil-containing solutions (0.1 gram of oil added per ml.) by the proposed method also gave the same values.

Analyses of petroleum ether solutions containing carotene, oxidized carotene, xanthophyll, and oil, are shown in Table I, samples 6 and 7. Analyses of these samples, using the aluminum oxide column, gave the same carotene content as was obtained by the analyses of similar solutions containing no oil, using the dibasic calcium phosphate column. The oil, therefore, did not interfere with the removal of these noncarotene chromogens by aluminum oxide when the analyses were made as described.

Table II. Comparison of Aluminum Oxide Column for Removal of Noncarotene Chromogens with Methods of Peterson and Moore

(Petroleum ether extract of dehydrated alfalfa leaf meal that had been stored a year. Micrograms of carotene in 10-ml. aliquot)

Al_2O_3 Column, Triplicate	Moore Method, Triplicate	Peterson Method, Triplicate
180, 180, 179	182, 185, 185	... ...
	1 gram of oil added to aliquots	
180, 181, 180	... ...	207, 218, 214

Comparison with Moore and Peterson Methods

A sample of alfalfa that had been in storage for almost a year was extracted with petroleum ether, and 10-ml. aliquots of the solution were analyzed for carotene by the proposed method and by passage through a column of dibasic calcium phosphate. The results by the two methods were very similar. Aliquots of 10 ml., to which 1 gram of refined corn oil was added, were analyzed by the proposed method and by the Peterson method (*9*). The method of Moore was not used because it is not directly applicable to solutions containing such large amounts of oil. The Peterson method gave appreciably higher results than the aluminum oxide method. Results with the latter agreed with the analyses made before the addition of the oil. The data are given in Table II. It has been previously shown (*1*, *2*, *5*, *7*) that methods based on phasic separation do not remove all noncarotene chromogens.

Cottonseed oil solutions of carotene that had been in storage under various conditions were analyzed (1) by simply diluting with petroleum ether to a suitable volume, (2) by the

Table III. Comparison of Proposed Method with Dilution and Peterson Methods

(Samples consist of cottonseed oil solutions of crystalline carotene after 7 months' storage under various conditions. Micrograms per 1 ml. of oil)

Sample No.	Apparent Carotene, Obtained by Dilution Only, Duplicates	True Carotene Proposed method, duplicates	True Carotene Peterson method, duplicates
S 127	171, 170	151, 157	156, 155
S 128	173, 175	160, 158	156, 160
S 129	175, 177	161, 160	160, 158
S 130	198, 199	175, 176	175, 176
S 132	198, 198	175, 175	175, 175
S 138	155, 155	139, 139	139, 139
S 139	178, 178	163, 165	160, 165
S 140	186, 186	170, 170	176, 176

proposed method, and (3) by the method of Peterson. The data, given in Table III, indicate that colored oxidation products of carotene may develop during the storage of oil solutions of carotene and, therefore, analyses made without their removal may be in error. Very close agreement with the Peterson method was obtained in these samples, which were prepared with crystalline carotene (90 per cent beta and 10 per cent alpha) and refined cottonseed oil. As noted above, this agreement was not obtained in the analyses of oil containing the chromogens extracted from alfalfa that had been in storage for some time. It appears that the Peterson method is more efficient in removing colored oxidation products of carotene than it is in removing some of the other noncarotene chromogens that may be present in a plant extract.

Satisfactory analyses were also made by the proposed method on solutions of carotene in refined corn, refined soybean, raw coconut, and raw rice bran. It may be assumed that the method will be satisfactory for other vegetable oils.

Summary

A rapid method is described for the determination of carotene in vegetable oil solutions without saponification. The colored oxidation products of carotene that develop during storage are removed by this method. The carotene is separated from the other chromogens by passing a petroleum ether solution of the oil containing the mixed chromogens through a Tswett column of aluminum oxide and eluting the carotene with 2 per cent acetone in petroleum ether. The method will also separate carotene from the pigments extracted by petroleum ether (30° to 60° C.) from dehydrated alfalfa, as well as from very large amounts of xanthophyll. The volume of sample and eluent must be controlled to ensure accurate separations of carotene by the column of aluminum oxide prepared as described.

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Detecting Adulteration of Ethylvanillin with Vanillin

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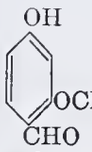
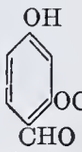
Vanillin,  , and ethylvanillin,  , would be expected to behave alike in chemical reactions because of their similar structures. Their main functional groups, —OH and —CHO, are identical, and their less active functional groups, —OCH_3 and —OCH_2CH_3, differ only by one —CH_2 grouping. Tests for distinguishing between them are therefore not very common, much less those for detecting the presence of one in the other. In fact, only one really satisfactory test has been published (*2*) for detecting ethylvanillin in the presence of vanillin, and none for detecting vanillin in the presence of ethylvanillin.

The two compounds, together with coumarin, are widely used in the manufacture of imitation vanilla flavors. The compound used must be stated on the label of the product, and if only vanillin is claimed by the label, the presence of ethylvanillin in the mixture must be considered adulteration. However, because ethylvanillin is much more expensive than vanillin and three times as much vanillin as ethylvanillin must be used to produce comparable products, adulteration of this type is not to be expected. When ethylvanillin is declared on the label, federal specification EE-E-911a calls for at least 0.33 gram of that substance per 100 cc. of imitation vanilla flavor. It would then be very profitable to substitute vanillin for some ethylvanillin.

Testing laboratories which examine imitation vanilla flavor for prospective purchase of the merchandise could use the following procedure to test for adulteration of ethylvanillin with vanillin.

Reagents

1. Two per cent alcoholic solution of potassium hydroxide, to which excess ammonium thiocyanate has been added.
2. A mixture of 1 cc. of 7 per cent aqueous cupric sulfate pentahydrate, 4 cc. of 3 per cent alcoholic ferric chloride dodecahydrate, and 5 cc. of 95 per cent ethyl alcohol.

Procedure

The ethylvanillin-vanillin fraction of the product is extracted with alcohol-free ether, and if not crystalline is purified according to the method of the A. O. A. C. (*1*). Twenty-five milligrams of the residue, dried in a sulfuric acid desiccator, are placed in a medium-sized test tube. For comparative purposes, 25 mg. of pure ethylvanillin may be placed in another test tube and put through the same procedure as the unknown. Three to 4 cc. of 95 per cent ethanol are added to the solid and the tube is shaken. A drop of reagent 1 and then a drop of reagent 2 are added and the mixture is gently shaken for a minute. In the presence of vanillin a red color is produced, which fades very slightly during several hours. If only ethylvanillin is present, a light yellow color will result from a very rapidly fading reddish solution.

The test tubes used must be perfectly clean and it is advisable to rinse them with alcohol before the test. If a quantitative es-

timate of the amount of vanillin present is desired, the color produced by the unknown may be compared in the colorimeter with those produced by known mixtures of vanillin and ethylvanillin during the same period of time.

The method has been found to detect the presence of at least 1 mg. of vanillin in the specimen taken for analysis. Coumarin does not interfere. Fresh reagents should be prepared after 2 days, since too much water and the tendency of reagent 2 to separate will cause a decrease in the sensitivity of the test. The procedure must be carefully followed, since the addition of too much reagent or of reagent 2 before reagent 1 may lead to erroneous results.

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Analysis of Constant-Pressure Filtration Data

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RUTH, Montillon, and Montonna (*1*) developed the equation

$$(V + C)^2 = K(\theta + \theta_0) \quad (1)$$

relating the volume, V, of filtrate produced during a given filtration at constant pressure, to the filtering time θ, the terms C and θ_0 representing, respectively, the theoretical amount of filtrate and filtering time required to develop the initial press resistance. To evaluate the constants of this equation they plotted $d\theta/dV$ against V, securing the straight line

$$d\theta/dV = 2(V + C)/K \quad (2)$$

the slope and V-intercept of which determine K and C, from which θ_0 is found by substitution in Equation 1. They showed how the determination of the constants may further be simplified by the assumption of a parabolic V-θ relation, permitting determination of the constants from the data of any three instants during the filtration.

Herewith is presented an alternate method of evaluating the filtration constants, applicable to any filtration represented by Equation 1. The working assumptions are that at $\theta = 0$, $V = 0$, and that the quantities of filtrate are V_1 and V_2 at times θ_1 and θ_2, with θ_2 chosen to be twice θ_1.

Substitution of these conditions successively in Equation 1 gives

$$C^2 = K\theta_0 \quad (3)$$
$$(V_1 + C)^2 = K(\theta_1 + \theta_0) \quad (4)$$
$$(V_2 + C)^2 = K(\theta_2 + \theta_0) \quad (5)$$

Subtraction of Equation 3 from 4 and 5 and substitution of nV_1 for V_2 give, following some manipulation,

$$C = V_1(n^2 - 2)/2(2 - n) = F_1V_1 \quad (6)$$
$$K = V_1^2n(n - 1)/\theta_1(2 - n) = F_2V_1^2/\theta_1 \quad (7)$$
$$\theta_0 = \theta_1(n^2 - 2)^2/4n(2 - n)(n - 1) = F_3\theta_1 \quad (8)$$

The relations between F_1, F_2, and F_3 and n are shown in Figure 1 for $1.2 < n < 1.9$, with the inset covering the values for n in the neighborhood of $\sqrt{2}$.

To evaluate the constants of any constant-pressure filtration by the use of this method it is necessary to know the amounts of filtrate at two elapsed times of filtration for which $\theta_2 = 2\theta_1$. The ratio of the two values of V gives n, from which the quantities F can be found for substitution in Equations 6, 7, and 8 for direct determination of C, K, and θ_0.

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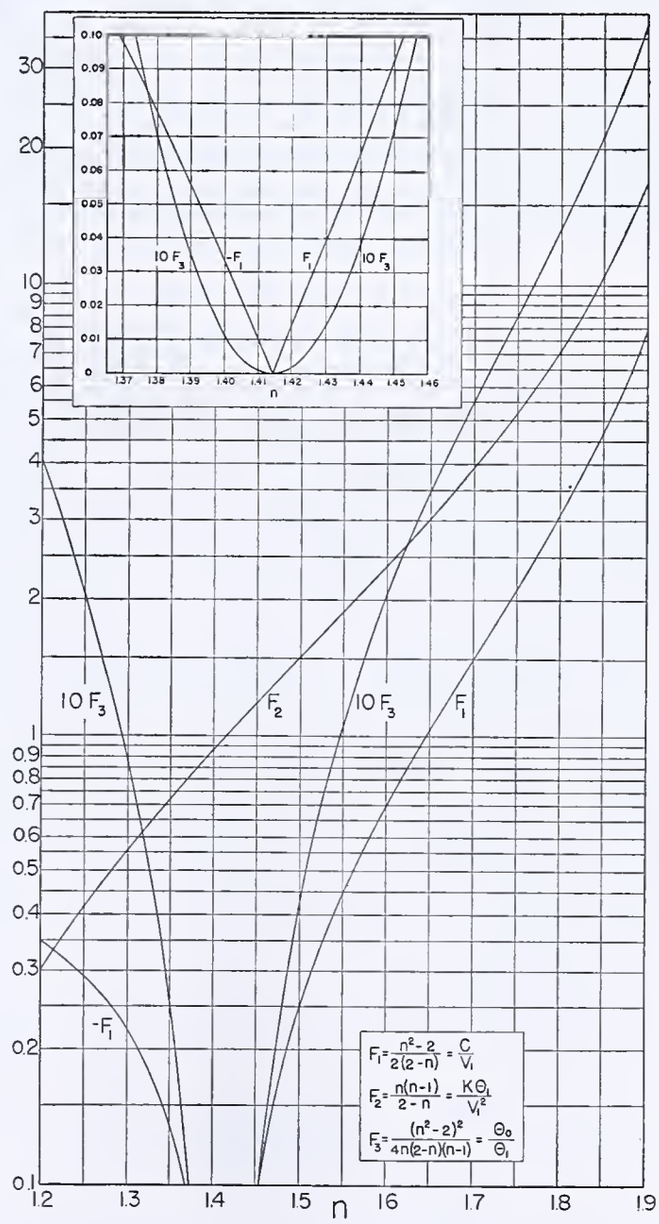

Figure 1
Filtration Constants, F, from Values of n

The Chemistry of Indium

A Colorimetric Method for the Estimation of Small Amounts of Indium

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THE suitability of 8-hydroxyquinoline as a reagent for the estimation of small amounts of indium was pointed out by Geilmann and Wrigge (*5*), who reported that quantitative precipitation of the compound $In(C_9H_6ON)_3$ could be effected at 70° to 80° C. from a sodium acetate–acetic acid buffer, and indium then determined either by direct weighing of the precipitate after drying at 120° or by application of the usual bromometric titration procedure after solution of the compound in warm 10 to 15 per cent hydrochloric acid. Accurate results in the concentration range 0.83 to 70.8 mg. of indium were obtained.

The possibility of utilizing the 8-hydroxyquinoline derivative of indium for the colorimetric estimation of the element has, apparently, escaped consideration. The compound $In(C_9H_6ON)_3$ dissolves readily in chloroform (a not unexpected fact, since chelate linkages within the molecule completely satisfy the coordination number of six which is normally shown by indium) yielding bright yellow solutions, the colors of which are of sufficient intensity to be readily detectable even at indium concentrations below 1 mg. per liter of solvent. Consequently, it seemed advisable to investigate this possibility, and the results here reported summarize a spectrophotometric examination of the system and a procedure for the extraction of the metal and its subsequent colorimetric estimation as the 8-hydroxyquinoline complex.

Apparatus and Materials

All colorimetric measurements reported herein were made with a Cenco-Sheard spectrophotelometer (*9*), using cells of 1-cm. (±0.5 per cent) thickness and employing a slit width of 5 mμ. Measurements of pH were made with a Beckman Laboratory Model G pH meter, the glass electrode of which was calibrated against a standard phthalate buffer.

A standard indium sulfate solution containing 11.64 mg. of indium per milliliter as determined gravimetrically by hydroxide precipitation (*8*) was prepared from anhydrous sulfate obtained originally from a sample of the metal of 99.98 per cent purity (*8*). From one portion of this solution, the indium derivative of 8-hydroxyquinoline was prepared according to the directions of Geilmann and Wrigge (*5*). The washed and dried product analyzed 20.81 per cent indium as compared with 20.97 per cent calculated for $In(C_9H_6ON)_3$. From a second portion of the sulfate solution, a stock indium solution containing the equivalent of 1 mg. of the metal in 10 ml. was obtained by dilution.

Chloroform, containing 1 per cent ethanol by volume, was of analytical reagent quality. During the investigation, it was found convenient to recover chloroform containing 8-hydroxyquinoline or its indium derivative by shaking with 6 *N* sulfuric acid, which completely extracts these materials, distilling from lime, and adding ethanol in the same fashion as recommended for recovery from dithizone extractions (*2*). Chloroform so recovered was colorimetrically indistinguishable from the original material. Indium can be recovered after destruction of the quinoline material (*5*).

The 8-hydroxyquinoline used was an Eastman Kodak preparation, and other chemicals were of the best qualities obtainable. Gallium and thallic sulfate solutions were obtained from the pure oxides.

Spectrophotometric Examination of Chloroform Solutions of the Indium Derivative of 8-Hydroxyquinoline

Spectral transmittancy studies over a wave-length range of 350 to 700 mμ were made upon solutions prepared by dilution to appropriate concentrations of a stock solution containing the indium derivative of 8-hydroxyquinoline in quantity corresponding to 1000 mg. of indium per liter of chloroform. Characteristic data are given in Figure 1.

Measurements at concentrations greater than 50 mg. of indium per liter are not included, but the curves obtained differ only in a marked broadening of the absorption band. Maximum absorption is noted at 395 mμ for most samples, with a tendency to shift to 400 mμ at higher concentrations. Inasmuch as this absorption band is rather broad, differences in transmittancy between 395 and 400 mμ are in no instance particularly great. Because of this fact and because 400 mμ represents the lower limit on some instruments, all further measurements were made at 400 mμ.

Extraction and Colorimetric Estimation of Indium

Indium, like aluminum and ferric iron (*1*), can be extracted from aqueous salt solutions by shaking with a chloroform solution of 8-hydroxyquinoline. The procedure followed in all extractions amounted to vigorous shaking of a 25-ml. aqueous solution of the indium salt with four successive 5-ml. portions of a 0.01 *M* solution of 8-hydroxyquinoline in chloroform. The combined extracts were then diluted to 50 ml. with chloroform before colorimetric examination. At optimum pH values, usually the third, and always the fourth, extract appeared colorless.

The marked effect of pH upon precipitations with 8-hydroxyquinoline (*3*, *4*, *6*) is also apparent in these extractions (*1*). The most suitable pH for complete extraction of indium was found by extracting solutions containing the equivalent of 20 mg. of indium per liter of chloroform and adjusted

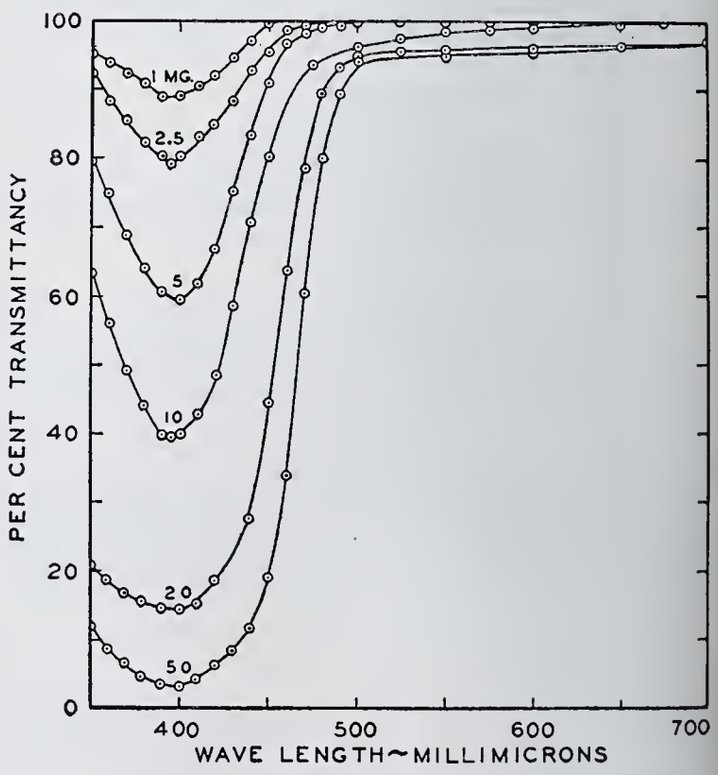

FIGURE 1. TRANSMITTANCY CURVES FOR INDIUM DERIVATIVE OF 8-HYDROXYQUINOLINE IN CHLOROFORM

Table I. Estimation of Indium in Indium Sulfate Solutions

No.	Mg. of Indium per Liter of Chloroform Taken	Found	Deviation
1	0.10	0.20	+0.10
2	0.30	0.30	0
3	0.70	0.60	−0.10
4	1.50	1.45	−0.05
5	4.00	4.00	0
6	8.00	7.90	−0.10
7	12.0	11.9	−0.1
8	16.0	16.0	0
9	20.0	20.0	0
10	24.0	23.5	−0.5
11	32.0	33.8	+1.8

to various pH values by the addition of either 0.1 *N* sodium acetate or sulfuric acid, determining the per cent transmittancies at 400 mμ, and comparing these values (through the use of a calibration curve) with the sample showing maximum absorption. In this manner, the degree of extraction was obtained at each pH value.

Reference to the plot of these results given in Figure 2 indicates that while extraction begins slightly above pH 1.9, it is complete only at pH 3.2. Complete removal of indium occurs within the pH range of 3.2 to 4.5, and in all subsequent extractions a pH of 3.5 was employed.

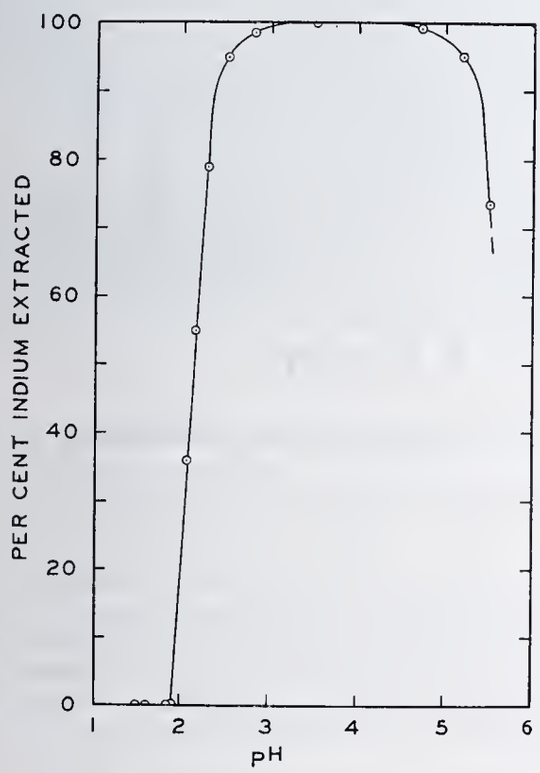

Figure 2. Effect of pH upon Extraction of Indium

The pH values given in Figure 2 are for the aqueous solutions before shaking with the 8-hydroxyquinoline reagent. During the extraction process, some of the amine enters the aqueous phase with an attendant increase in pH, but under standard conditions of extraction, the initial pH appears to be the more readily attainable and therefore the more important. When the pH is 1.9 or less, 8-hydroxyquinoline is completely extracted from the chloroform owing to combination with the acid, and at pH values above 4.5, precipitation of hydrous indium hydroxide both decreases the amount of extractable indium and renders layer separation difficult through emulsification of water in chloroform.

Plotted in Figure 3 are data obtained from transmittancy studies at 400 mμ upon extracts made at pH 3.5 from indium sulfate solutions of varying concentrations prepared from the stock solution containing 1 mg. of indium in 10 ml. Beer's law is obeyed at concentrations up to 18.0 mg. of indium per liter of chloroform, but at larger concentrations deviations become increasingly great. Intersection of the curve with the zero axis of concentration at a point below that corresponding to 100 per cent transmittancy is due to the fact that a chloroform solution of 8-hydroxyquinoline, although apparently colorless, shows a slight absorption at 400 mμ (*1*, *7*). Use of constant conditions of extraction obviates this difficulty.

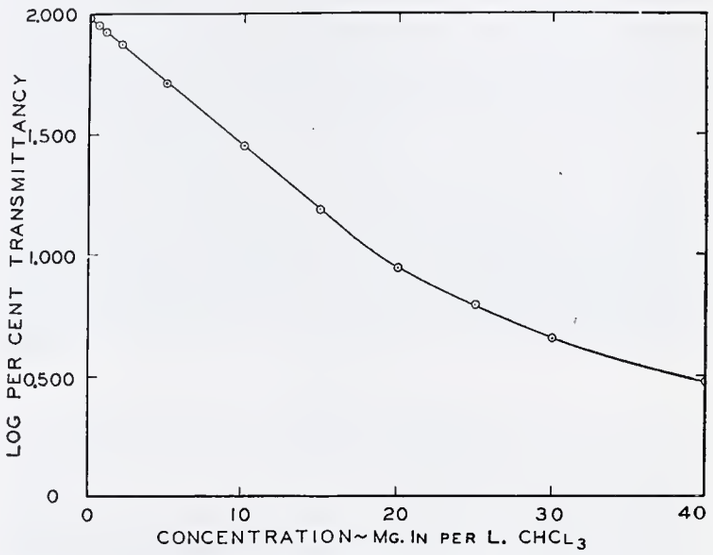

Figure 3. Relation between Transmittancy and Indium Concentration

The reproducibility and accuracy of the method are indicated by the data in Table I. In the range of 0.30 to 20.0 mg. of indium per liter of chloroform, deviations are small, but at higher and lower concentrations they become marked. If concentrations be expressed in terms of actual indium content instead of per liter of chloroform, the sensitivity of the method is apparent. Since given concentrations were obtained from 50-ml. chloroform solutions, accurate measurements resulted with actual indium contents in the range 0.015 to 1.00 mg.

Interferences

Nitrate, chloride, or sulfate solutions containing in 25-ml. portions the equivalents of 20 mg. of various metals per liter of chloroform (on the basis of complete extraction) were extracted at pH 3.5 by the above procedure, transmittancies at 400 mμ being determined. The following ions were not extracted under these conditions: magnesium, calcium, strontium, zinc, cadmium, mercuric, stannic, lead, manganous, chromic, and silver. The following ions yielded extracts showing transmittancies below that for 8-hydroxyquinoline and were, therefore, extracted (at least in part): aluminum, gallium, thallic, stannous, bismuth, cupric, ferric, ferrous, nickel, and cobalt (slightly). Ions of the latter group would interfere with the estimation of indium by this method.

Estimation of indium should be possible in the presence of any ion not extractable at pH 3.5. In Table II are summarized data for determinations made in the presence of varying quantities of zinc, cadmium, and lead ions, concentrations again being expressed relative to 1 liter of chloroform. The feasibility of the procedure is apparent.

Since the most persistent impurity in indium is iron, a method for the estimation of indium in the presence of iron would be of value. Application of the present procedure

Table II. Estimation of Indium in Presence of Zinc, Cadmium, or Lead

(20.0 mg. of indium per liter of chloroform taken in all cases)

Impurity Added		Indium Found
Ion	*Mg.*	*Mg.*
Zn^{++}	20	20.0
	100	20.0
	200	20.0
Cd^{++}	20	20.0
	100	20.0
	200	20.0
Pb^{++}	20	20.0
	100[a]	20.0

[a] Some precipitation of lead sulfate noted.

would be feasible if it were possible to extract either ferric iron or indium to the exclusion of the other at an appropriate pH. However, since the extraction of the ferric derivative of 8-hydroxyquinoline is complete only at pH values above 1.9 to 2.0 (*1*, *7*) whereas extraction of indium begins in this pH interval, such a procedure would not appear to be suitable. Furthermore, the extensive absorption of the ferric complex at 400 mμ (*7*) would cause any iron unextracted at a pH lower than 1.9 to interfere with indium extracted at pH 3.5. Attempts at successive extractions of iron and indium have fully demonstrated the lack of reliability of such a procedure.

Summary

The 8-hydroxyquinoline derivative of indium dissolves readily in chloroform, yielding yellow solutions which show a fairly broad absorption band in the region of 395 to 400 mμ. At 400 mμ, such solutions obey Beer's law in concentrations up to 18.0 mg. of indium per liter of chloroform and lend themselves to the colorimetric estimation of the element.

Indium ion can be completely extracted from aqueous solutions in the pH range 3.2 to 4.5 by shaking with a chloroform solution of 8-hydroxyquinoline. Of the commoner ions, only the following are extracted at pH 3.5 and therefore interfere with the colorimetric estimation of indium in the chloroform extracts: aluminum, stannous, bismuth, ferric, ferrous, cobalt, nickel, and cupric. Gallium and thallic ions also interfere.

Indium can be estimated accurately in the presence of considerable quantities of zinc, cadmium, and lead ions, but it appears impossible to determine indium accurately in ferric-indium ion solutions by extraction of ferric iron at a low pH and subsequent extraction of indium followed by colorimetric examination.

The colorimetric procedure yields accurate results for indium concentrations in the interval of 0.3 to 20 mg. per liter of chloroform or 0.015 to 1.00 mg. of the metal in a 25-ml. aqueous solution. Analyses of samples containing larger amounts of indium should be possible through sufficient dilution of the chloroform extract.

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The Dead-Stop End Point

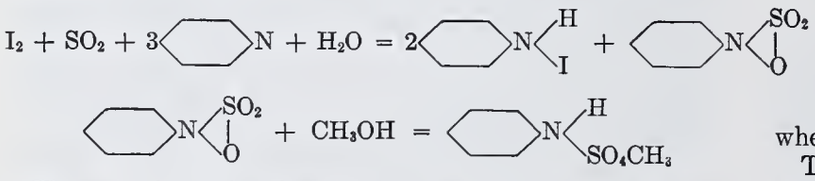
As Applied to the Karl Fischer Method for Determining Moisture

GRANT WERNIMONT AND F. J. HOPKINSON, Eastman Kodak Company, Rochester, N. Y.

The chemical reaction involved in the Karl Fischer method (*2*) for determining water was given originally as follows:

$$2H_2O + I_2 + SO_2.2C_5H_5N + 2C_5H_5N = (C_5H_5N)_2H_2SO_4 + 2C_5H_5N.HI$$

Smith, Bryant, and Mitchell (*4*) have shown that the reaction is not so simple as this and that it occurs in two distinct steps:



The second reaction does not involve water and this makes it necessary to control all conditions with care during the titration in order to get reproducible results. No equation can be written to represent the stoichiometric relation between the water consumed and the amount of Fischer reagent used up. This is not a serious drawback, however, because the Fischer reagent is readily standardized against pure water or a standard solution of water.

Fischer, as well as Smith, Bryant, and Mitchell, detected the end point visually by the appearance of brown iodine when all the water had reacted. This is often difficult because the products of the reaction are yellow and the brown color of iodine is not easily seen under these conditions. Starch does not serve as an indicator for iodine in these nonaqueous solutions.

Almy, Griffin, and Wilcox (*1*) used a potentiometric method with platinum and tungsten electrodes for detecting the end point which was more precise than the visual method. The authors have been able to duplicate their results, although the manipulation difficulties are a little greater than when the end point is detected visually.

The authors have found that the dead-stop method of Foulk and Bawden (*3*) gives a sharp and reproducible end point in these nonaqueous solutions with fewer manipulation difficulties than the potentiometric method and better precision than the visual method. It depends upon the fact that when an electromotive force of 10 to 15 millivolts is impressed upon two platinum electrodes immersed in the Fischer reagent, sufficient current flows through the solution to deflect a galvanometer off the scale. During the titration of the Fischer re-

FIGURE 1. TITRATION APPARATUS

agent with a standard solution of water in methanol, the galvanometer remains deflected until the end point is approached. Near the end point the galvanometer is affected by each addition of water solution, and at the end point the galvanometer suddenly comes to a steady reading near its rest point. The reverse titration—that is, the addition of Fischer reagent to a solution containing water—was found to be less satisfactory and was not used in these experiments.

The dead-stop apparatus is continuous-reading, and requires practically no adjustment from day to day, while the potentiometric apparatus must be adjusted before each individual titration.

Apparatus

The two standard solutions were dispensed from automatic burets, protected from atmospheric moisture by means of Drierite.

The apparatus for stirring the solutions was similar to that of Almy, Griffin, and Wilcox (*1*) and is shown in Figure 1. The stirrer, buret tip, and platinum electrodes pass through a Bakelite stopper which has been turned to fit a No. 27 standard taper, 250-ml., glass-stoppered Erlenmeyer flask. The stirring propeller and its bearing are made of stainless steel.

The dead-stop apparatus was similar to that used by Wernimont and Hopkinson (*6*).

A small weighing pipet for standardizing the solutions consisted of a 2-ml. medicine dropper with a rubber bulb and a Nichrome suspension wire.

Reagents

FISCHER REAGENT. A 700-ml. portion of pyridine, Eastman reagent No. 214-H, was measured into a 1-liter Erlenmeyer flask and tared on a platform balance. Gaseous sulfur dioxide was passed from a cylinder into the pyridine through a gas-dispersion tube until 380 grams had been added. When the solution had cooled to room temperature, 1 liter of pyridine, 200 ml. of anhydrous methanol, and 500 grams of resublimed iodine were added; it was immediately cooled in an ice bath and shaken occasionally until all the iodine was dissolved. The reagent was stored in a glass-stoppered bottle and dispensed from an automatic 50-ml. buret, protected from atmospheric moisture by means of Drierite.

STANDARD WATER SOLUTION. Twenty milliliters of distilled water were added to 3.8 liters of commercial methanol and this solution was dispensed from an automatic buret, protected from atmospheric moisture by means of Drierite, directly into the titration flask in the stirring apparatus. The ratio, R, between this solution and the Fischer reagent was first determined by direct titration to a dead-stop end point. Then 25-ml. portions of anhydrous methanol were titrated for their moisture content by adding Fischer reagent until the brown color of iodine appeared and back-titrating with standard water solution to a dead-stop end point. The milliliters of standard water solution, b, equivalent to the water in the methanol were calculated as follows:

$$b = (\text{ml. of Fischer reagent} \times R) - (\text{ml. of standard water solution})$$

Finally, 0.13- to 0.15-gram portions of water were weighed from a small weighing pipet into dry Erlenmeyer flasks containing 25 ml. of anhydrous methanol. Fischer reagent was then slowly added to these solutions until an excess was present and the excess was immediately back-titrated with standard water solution to a dead-stop end point. The water factor, W, of the standard water solution was calculated as follows:

$$W = \frac{\text{grams of water weighed}}{(\text{ml. of Fischer reagent} \times R) - (\text{ml. of standard water solution}) - b}$$

An alternative method for standardizing the solutions and making the analysis was devised after this work was completed which made the calculations somewhat simpler. The distilled water was weighed into Erlenmeyer flasks containing 25 ml. of anhydrous methanol. Several extra flasks containing 25 ml. of methanol were also set up to serve as blanks. Exactly 25.00 ml. of Fischer reagent were added to the first blank and titrated with the standard water solution to a dead-stop end point. The remaining blanks were titrated in a similar manner, using the same volume of Fischer reagent; if they agreed within 0.1 ml., the water standards were then titrated. The difference between each standardization titration and the average titration of the blanks is equivalent to the amount of water weighed out. The water factor, W, of the standard water solution in this case is:

$$W = \frac{\text{grams of water weighed}}{\text{net ml. of standard water solution}}$$

This procedure automatically corrects for the water in the solvents used to dissolve the standards or samples and it has been most satisfactory when the samples all have about the same water content.

ANHYDROUS METHANOL. Commercial methanol was allowed to stand over Drierite for several days and was then distilled from Drierite and collected in a receiver, protected from atmospheric moisture by means of Drierite. It usually contained 0.03 to 0.05 per cent of moisture as determined by this method on a 25-ml. sample.

Methanol which is to be used as a solvent for analyzing traces of moisture in other materials should be as dry as possible, or the uncertainty of the blank determinations becomes appreciable.

Stability of the Standard Solutions

The stability of the two standard solutions was tested by determining the ratio, R, between them at various intervals and checking the water factor, W, of the standard water solution against weighed amounts of water. The results, given in Table I, are the average of four to five determinations. The range of the individual determinations never exceeded 6 parts per thousand and the average deviation was about 2 parts per thousand. The factor, W, remained constant within experimental error; but it was necessary to determine the ratio, R, whenever the solutions were used.

Table I. Stability of Reagents

Date	R^a	W^b
1–5–40	1.106	0.00524
1–8–40	1.030	..
1–9–40	1.020	..
1–11–40	0.984	..
1–17–40	0.921	0.00523
2–16–40	0.919	0.00526

[a] R = ml. of standard water solution per ml. of Fischer reagent.
[b] W = grams of water per ml. of standard water solution.

Experimental Results

The precision and accuracy of determining water in the presence of methanol were determined by weighing various amounts of water from a small weighing pipet into Erlenmeyer flasks containing 10 ml. of anhydrous methanol. The resulting solutions and the original solvent were analyzed for their water content by adding Fischer reagent until the brown color showed that an excess was present. The excess was immediately titrated with standard water solution to a dead-stop end point. The results given in Table II, show that the recovery of water was satisfactory. Similar experiments in which the methanol was replaced by acetone gave the results shown in Table III. In every case the agreement between water found and water present is satisfactory up to about 200 mg. of water.

Table II. Analysis of Water in Presence of 10 Milliliters of Methanol

Sample No.	Fischer Reagent Added	Standard Water Solution Back-Titrated	Standard Water Solution, Net[a]	Water Found	Water Present[b]	Difference
	Ml.	*Ml.*	*Ml.*	*Mg.*	*Mg.*	*Mg.*
1	5.00	6.20	0.70	3.74	3.74	...
2	5.00	6.20	0.70	3.74	3.74	...
3	8.00	6.60	4.43	23.7	23.3	+0.4
4	8.00	6.40	4.63	24.7	24.4	+0.3
5	10.00	4.55	9.24	49.3	50.1	−0.8
6	15.00	8.30	12.38	66.1	65.5	+0.6
7	15.00	6.85	13.83	73.9	74.0	−0.1
8	20.00	9.05	18.53	99.0	99.1	−0.1
9	20.00	4.20	23.38	124.8	125.6	−0.8
10	25.00	7.55	26.93	143.8	144.3	−0.5
11	25.00	6.00	28.48	152.1	152.5	−0.4
12	30.00	5.70	35.67	190.5	191.6	−1.1

R = 1.379 ml. of standard water solution per ml. of Fischer reagent.
W = 0.00534 gram of water per ml. of standard water solution.

[a] (Ml. of Fischer reagent × R) − ml. of standard water solution back-titrated.
[b] Sum of water added to and found in 10 ml. of methanol.

Table III. Analysis of Water in Presence of 10 Milliliters of Acetone

Sample No.	Fischer Reagent Added	Standard Water Solution Back-Titrated	Standard Water Solution, Net[a]	Water Found	Water Present[b]	Difference
	Ml.	*Ml.*	*Ml.*	*Mg.*	*Mg.*	*Mg.*
1	5.00	5.00	1.65	8.8	8.6	...
2	5.00	5.10	1.55	8.3	8.6	...
3	5.00	3.00	3.65	19.5	21.7	−2.2
4	5.00	2.90	3.75	20.0	21.4	−1.4
5	10.00	6.10	7.20	38.4	38.1	+0.3
6	10.00	5.50	7.80	41.7	42.2	−0.5
7	15.00	9.55	10.40	55.5	54.2	+1.3
8	15.00	9.05	10.90	58.2	57.0	+1.2
9	15.00	6.45	13.50	72.1	72.1	0.0
10	15.00	6.05	13.90	74.2	77.7	−3.5
11	20.00	7.40	19.20	102.5	106.2	−3.7
12	20.00	4.50	22.10	118.0	117.1	+0.9
13	25.00	5.80	27.45	146.6	148.9	−2.3
14	30.00	8.20	31.70	169.3	168.7	+0.6

R = 1.330 ml. of standard water solution per ml. of Fischer reagent.
W = 0.00534 gram of water per ml. of standard water solution.

[a] (Ml. of Fischer reagent × R) − ml. of standard water solution back-titrated.
[b] Sum of water added to and found in 10 ml. of acetone.

Table IV. Analysis of Known Solutions of Water in Methanol

Sample Weight	Fischer Reagent Added	Standard Water Solution Back-Titrated	Standard Water Solution, Net[a]	Water Found	Water Present
Grams	*Ml.*	*Ml.*	*Ml.*	%	%
19.8	10.00	6.90	2.29	0.061	0.059
19.8	10.00	6.80	2.39	0.063	
19.8	15.00	5.45	8.33	0.22	0.218
19.8	15.00	5.45	8.33	0.22	
7.92	15.00	5.80	7.98	0.53	0.525
7.92	15.00	5.75	8.03	0.53	
1.580	20.00	2.90	15.48	5.13	5.02
1.575	20.00	2.85	15.53	5.17	

R = 0.919 ml. of standard water solution per ml. of Fischer reagent.
W = 0.00524 gram of water per ml. of standard water solution.

[a] (Ml. of Fischer reagent × R) − ml. of standard water solution back-titrated.

Known solutions of water in methanol and in ethanol were made up by weighing various amounts of water from a small weighing pipet into 100-ml. volumetric flasks and making up to the mark with anhydrous solvent. Suitable amounts of these known solutions were then analyzed for their water content. The solvents were also analyzed and the "water present" column includes the water which came along with the solvent. The results, given in Tables IV and V, also show satisfactory recovery of water by this procedure.

The dead-stop apparatus and method have been used for the routine determination of moisture in lower alcohols, in 1,4-dioxane, and in mixtures of alcohols containing acetone up to about 15 per cent. It has not been used to determine water in mixtures of high acetone content because the acetyl chloride method of Smith, Bryant, and Mitchell (*5*) has been found more satisfactory in these cases. Some organic liquids, such as esters, hydrocarbons, and chlorinated hydrocarbons, do not dissolve readily in the Fischer reagent; but they have been analyzed with satisfactory results when mixed with anhydrous methanol.

Table V. Analysis of Known Solutions of Water in Ethanol

Sample Weight	Fischer Reagent Added	Standard Water Solution Back-Titrated	Standard Water Solution, Net[a]	Water Found	Water Present
Grams	*Ml.*	*Ml.*	*Ml.*	%	%
19.8	20.00	5.15	10.25	0.29	0.28
19.8	19.15	4.70	10.06	0.28	
7.92	31.00	4.85	19.02	1.35	1.35
7.92	32.85	6.15	19.14	1.36	
1.610	36.50	6.20	21.90	7.63	7.52
1.610	35.40	5.40	21.86	7.62	

R = 0.770 ml. of standard water solution per ml. of Fischer reagent.
W = 0.00561 gram of water per ml. of standard water solution.

[a] (Ml. of Fischer reagent × R) − ml. of standard water solution back-titrated.

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Modification of Cenco Spectrophotometer

Permitting Measurements of Reflection and Fluorescence Spectra

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METHODS for the determination of color of opaque objects fall, essentially, into three categories: (1) those employing color charts or standards for comparison, (2) those employing colorimeters and comparators which synthesize colors similar to the colored samples, and (3) those employing spectrophotometers. Of these three classes of apparatus, only the spectrophotometers have the advantage of resolving color into fundamental physical units.

The investigator who does not have available a reflection spectrophotometer, but does have the Cenco type transmission spectrophotometer (*3*), may readily modify that instrument to permit the measurement of reflection spectra. Fluorescence spectra may also be measured with this instrument through the use of a nearly identical optical system. The required modifications are described in the present paper.

Reflection Measurements

The spectrophotometer is rotated 60° counterclockwise from its normal position when viewed from above, as shown in Figures 1 and 2. The spring plate which holds filters in front of the slit

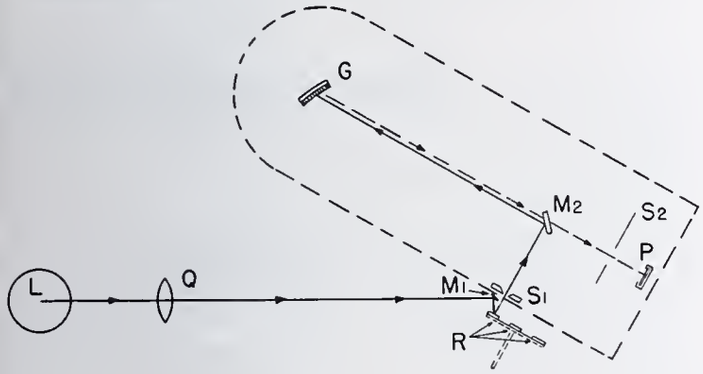

FIGURE 1. OPTICAL SYSTEM OF THE CENCO SPECTROPHOTOMETER AS MODIFIED FOR MEASURING REFLECTANCE SPECTRA

L, light source. *Q*, quartz lens. M_1, M_2, plane mirrors. *R*, samples for reflectance measurements. S_1, entrance slit. S_2, exit slit. *P*, photocell. *G*, grating

is removed and a back-silvered mirror (1 × 2 cm.) is attached at the side of the slit nearer the light source at an angle of approximately 37.5° to the optical bench. By means of the collimating lens, the image of the lamp filament is brought to a focus 1 cm. after reflection from the mirror. At this point the light strikes the objects from which reflection measurements are to be made. These solids, along with the magnesium oxide standard, are fastened by means of spring clips to a circular disk. This disk is rotated in a plane parallel to that of the entrance slit of the spectrophotometer, bringing successively the magnesium oxide standard and the individual samples into the reflecting position. Thus the light strikes the surface of the sample with an angle of incidence of 45°, and only the light reflected diffusely and in a direction normal to the surface passes through the monochromator to the photocell. The reflectance of the samples at the various wave-length scale settings is measured by the usual method in terms of the ratio of galvanometer deflections for the samples to those for the standard white.

The standard white reflector was prepared by pressing magnesium oxide powder into a circular pad in a Carver press, and then roughening its surface with fine emery paper. Samples of finely divided materials may also be prepared for color measurements by pressing them into a similar pad. In this form they are readily attached to the sample disk.

In order to obtain sufficient intensity of reflected light upon the photocell, using the lens furnished with the instrument, an entrance slit width of 2.5 mm. is used with an exit slit width of a nominal value of 20 mμ. In the region of maximum photocell sensitivity (approximately 585 mμ), a galvanometer deflection of 45 mm. was obtained for the standard. At wave lengths shorter than 430 mμ and longer than 660 mμ, deflections of less than 10 mm. are obtained and as deflections decrease the per cent error in reading the galvanometer increases. If the effective aperture of the collimating lens system is increased by the introduction of a second component similar to that furnished with the instrument, the intensities on the photocell are increased about 2.5 times. (The theoretical fourfold increase is difficult to attain because of space limitations.) This added intensity permits the extension of the effective range of the spectrophotometer to 400 mμ in the blue and 700 mμ in the red region of the spectrum, and permits the reduction of the exit slit to 10 mμ in the central portion of the range.

When a disk holding three samples and the magnesium oxide standard is used and readings are taken at 10-mμ intervals, the data for three samples may be obtained in less than an hour. Calculation of the data requires an additional half hour.

The curves in Figure 3 show the reflectance spectra of colored papers and make possible a comparison of results with those obtained upon a Hardy recording spectrophotometer. (The colored papers upon which the authors' measurements were made and reflection spectra data with which they are compared have been reproduced by the General Electric Co., *2*.)

Fluorescence Measurements

Fluorescence spectra are measured with the same optics, except that a General Electric (H-6) water-cooled mercury arc replaces the tungsten lamp as a source of excitation and an absorption cell containing the fluorescing solution replaces the reflecting samples. However, in this case, the intensity of fluorescence as a function of wave length is desired, rather than the intensity related to a standard reflector as above. Since the sensitivity of the photocell varies considerably with wave length, it was necessary to correct the instrument to a constant sensitivity value. A correction factor for the spec-

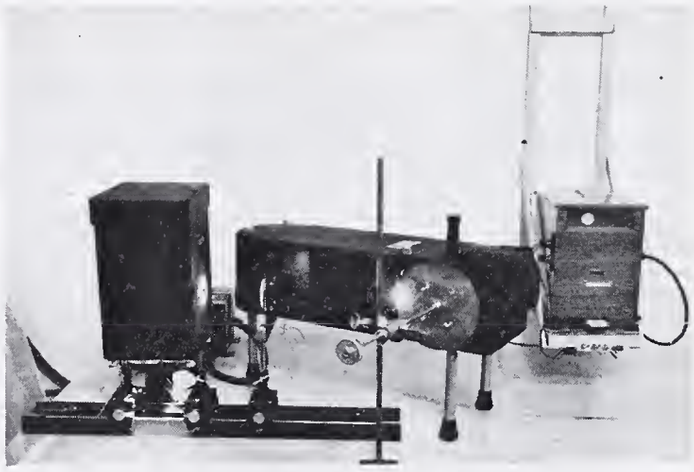

FIGURE 2. CENCO SPECTROPHOTOMETER AS MODIFIED FOR MEASURING REFLECTANCE SPECTRA

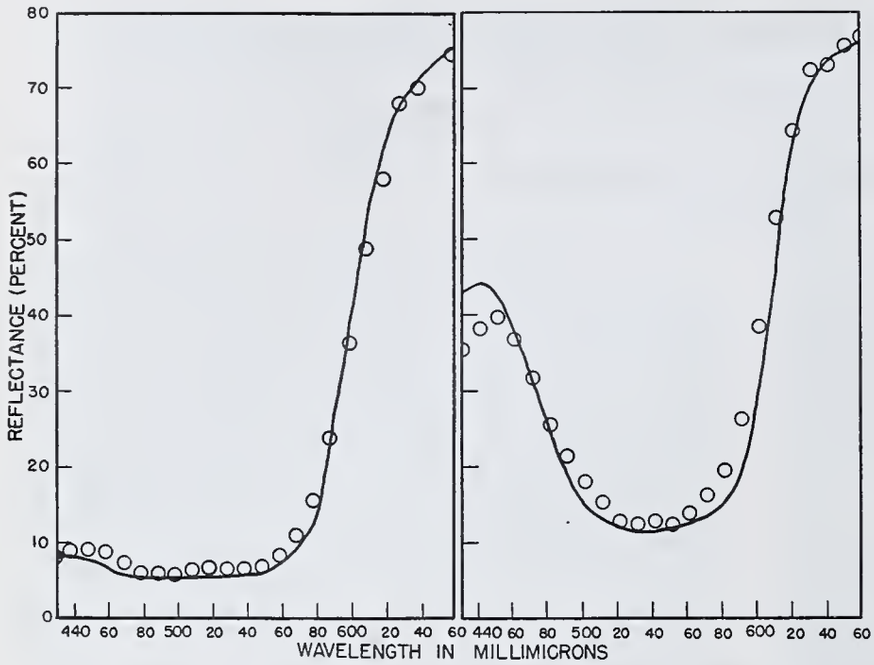

FIGURE 3. REFLECTANCE SPECTRA OF COLORED PAPERS
Circles represent data obtained with modified Cenco spectrophotometer. Curves are those recorded by G. E.-Hardy spectrophotometer

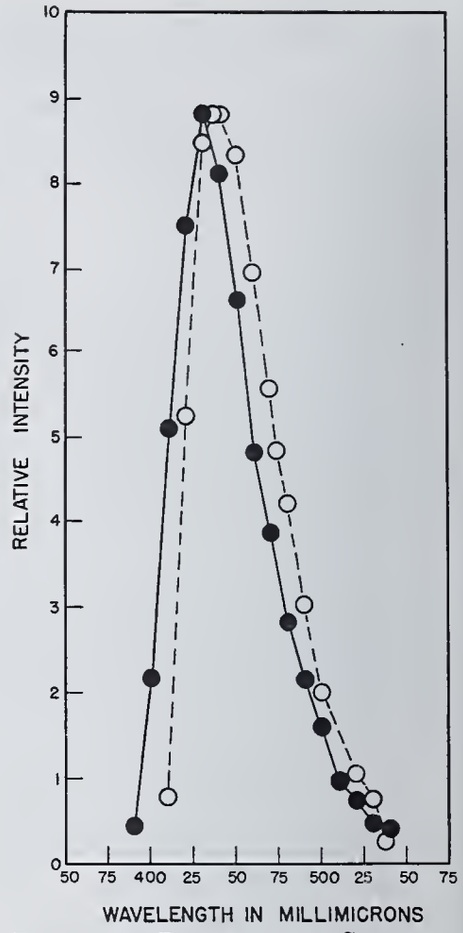

FIGURE 4. FLUORESCENCE SPECTRUM OF THIOCHROME
Solid curve, fluorescence received at approximately 180° to exciting beam. Broken curve, fluorescence received in line with exciting beam

trophotometer at each wave length was obtained by dividing the intensity of light isolated by the spectrophotometer from a tungsten source radiating at 3250° K. by a value calculated from Planck's equation for the intensity of light emitted at that wave length from a black body radiator at the same temperature.

The optical system is characterized by an arrangement in which the fluoresced light is observed from the direction of the source. A system which has been used frequently for exciting fluorescence utilizes a beam of exciting light 90° from the line of observation of the fluoresced light (*4*). Another arrangement is that of observing the fluoresced light in line with the exciting light through appropriate filters which remove the exciting light but transmit the fluorescence (*1*). In the two latter arrangements it can be shown algebraically, and it has also been observed experimentally (*5;* see also Figure 4), that the shape of the curve representing the spectral distribution of energy and position of the maximum vary with the concentration of the fluorescing substance as a result of reabsorption of the fluoresced light of shorter wave lengths. In the present arrangement in which the fluoresced light is observed from the direction of the source, the position of the maxima and shape of the curve are independent of concentration. The algebraic description of this arrangement is as follows:

Consider the intensity of fluoresced light of wave lengths between λ and $\lambda + d\lambda$ coming from an elementary volume, $dx\,dy\,dz$, in a fluorescing solution of infinite depth and excited by monochromatic light of incident intensity (I_0) uniform over the incident surface. Let the angle of the path of fluoresced light be 180° with respect to the exciting beam and 90° with respect to the plane of the window of the containing cell. When several constants (the quantum efficiency factor for the wave-length band $d\lambda$, the specific absorption coefficient of the solution for the exciting light, and the ratio of the energy values of the quanta corresponding to the wave lengths of exciting and fluoresced light) involved in the excitation of fluorescence are grouped into a single constant, K, the intensity of the fluoresced light, dI_f, reaching the measuring instrument from the elementary volume, $dx\,dy\,dz$, may be expressed as the product of (1) constant K, (2) the concentration of the fluorescing substance, (3) the intensity, ($I_0e^{-\alpha_a cx}$), of exciting light reaching depth x, and (4) the fraction of the intensity of fluoresced light transmitted back in the direction of the exciting beam through x cm. of solution ($e^{-\alpha_f cx}$):

$$dI_f = K\,c\,I_0e^{-\alpha_a cx} \times e^{-\alpha_f cx}\,dx\,dy\,dz$$

where α_f = specific absorption coefficient for fluoresced light
α_a = specific absorption coefficient for activating (exciting) light
c = concentration of fluorescing material in grams per liter
x = depth in cm.

Upon integrating this function with respect to x between the limits $x = 0$ and $x = \infty$ and substituting for $\int\int dy\,dz$ its equivalent, the cross-sectional area, A,

$$I_f = \frac{K}{\alpha_a + \alpha_f} \times I_0A$$

the concentration factor cancels out and therefore the intensity of the fluoresced light of any given wave length is independent of concentration.

In practice, an absorption cell of 1-cm. depth was used instead of the theoretical cell of infinite depth and the concentration of the fluorescent material was increased to give total absorption of the exciting light in a depth of 1 cm. In certain cases—e. g., that of thiochrome described below—90 per cent of the exciting light was absorbed in passage through the first millimeter of solution.

An example of the type of data taken with the apparatus and optical system just described is given in Figure 4 as the solid curve—the fluorescence spectrum of thiochrome (the alkaline oxidation product of vitamin B_1). The dotted line represents data taken with the optical system in which fluorescence is observed in line with the exciting beam. As explained above, the shift of the maximum toward the longer

wave lengths is due to the reabsorption of the shorter wave lengths of fluoresced light.

Summary

A simple modification of the Cenco spectrophotometer permits the measurement of reflection and fluorescence spectra with moderate resolution. The arrangement whereby fluorescence is observed from the same direction as the exciting light is superior to other possible arrangements, in that the shape of the curve representing the spectral distribution of energy and the position of the maximum are independent of concentration of the fluorescing substance.

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Ultraviolet Photometer for Analysis of Solutions

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A simple and inexpensive ultraviolet photometer, suitable for the analysis of solutions, is outlined in detail. Results obtained with phenol, sulfanilamide, and potassium nitrate are described, and applications to other substances, particularly aromatic compounds, are discussed.

MANY instruments and methods of analysis based on the absorption of light have been described in the literature of the past few years. Photometric procedures have been found to be very convenient, particularly in routine testing and control, because of their sensitivity and rapidity. A description of some common types of apparatus using visible light has been given recently by Müller (*6*).

Some instruments have also been developed to utilize the absorption of ultraviolet light to measure concentrations in solutions. Demarest (*2*), for example, has described a photometer which uses light of about 3300 Å. for the analysis of vitamin A. Similarly, Buswell and Dunlop (*1*), using a spectrograph and photographic plate, measured the concentrations of aqueous solutions of phenol by the absorption of radiation of approximately 2700 Å. This paper describes a photoelectric photometer which may be used in the analysis of solutions which absorb light of 2537 Å. The construction of such an apparatus has been facilitated recently by the development of inexpensive ultraviolet-sensitive photocells and high-silica, ultraviolet-transmitting glass. These have been combined with a commercial germicidal lamp, a convenient source of 2537 Å. radiation, to yield a simple instrument composed of readily available materials.

Instruments similar in principle to the one described in this paper have been developed by Woodson (*7*) and by Hanson (*4*) for the determination of concentrations of vapor in air.

Design and Operation of Apparatus

A schematic diagram of the optical and electrical system is given in Figure 1.

LIGHT SOURCE. A General Electric T-10 germicidal lamp was used in the manner described by Hanson (*4*). After a preliminary warm-up period of about an hour, the lamp maintained an intensity of radiation constant to within 0.1 to 0.2 per cent.

PHOTOELECTRIC DETECTOR. An RCA C-7032 phototube served as the light-sensitive element. The photocurrents were amplified by an RCA 954 acorn tube used in the manner described by Gabus and Pool (*3*), but in a somewhat modified circuit (*5*). With a potentiometer in the grid circuit and a galvanometer in the plate circuit, the amplifier can be used as a sensitive null point indicator, and difficulties due to nonlinearity in response may be eliminated. The changes in the plate resistance and the elimination of a separate battery for the photocell have increased the stability of the electrical system.

The light intensities are measured by the following procedure. With the shutter closed and the potentiometer, *P*, set at zero, the plate resistances are varied until the galvanometer shows a null reading. One of the absorption cells is then placed in the path of the light beam and the shutter is opened. The potential drop across the grid leak produced by the flow of the photocurrent through the high resistance is counterbalanced by increasing the potential imposed by the potentiometer until the plate meter has returned to its original reading. The reading of the potentiometer is then directly proportional to the intensity of the light incident on the phototube.

For a precision of 0.1 to 0.2 per cent, a Leeds & Northrup student potentiometer may be used to measure grid potentials. For less precise work a rheostat-potentiometer is suitable.

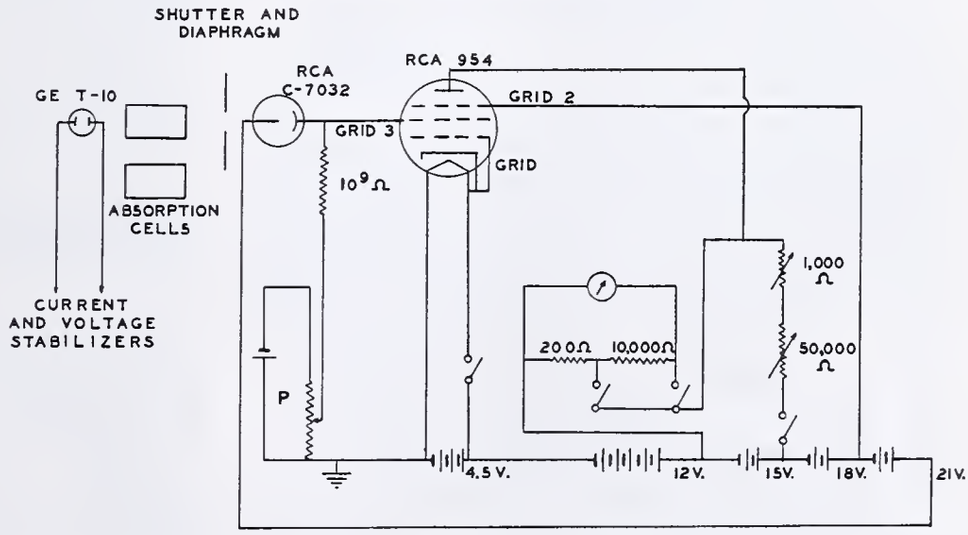

FIGURE 1. DIAGRAM OF ULTRAVIOLET PHOTOMETER

Absorption Cells. The body of the cells consisted of Pyrex tubing with suitable side arms for the introduction of solution. The windows, however, were cut from Corning No. 791 ultraviolet-transmitting glass, which transmits about 50 per cent of radiation of 2500 Å. wave length, and were sealed to the body of the cell by means of Sealstix cement.

The absorption cells were 10.02 cm. in length.

Calibration

To avoid frequent calibrations with mixtures of known composition, the author used the exponential relationship between light intensity and concentration

$$\log I_0/I = \epsilon\, c\, d \qquad (1)$$

where I_0 = intensity of light leaving pure solvent
I = intensity of light leaving solution
ϵ = extinction coefficient
c = concentration of solute
d = length of absorption cell

The transmission, I/I_0, was determined from the ratio of the potentiometer readings with solution and solvent, respectively, in the path of the light beam. With d and c known, one can calculate ϵ very readily.

The "effective" extinction coefficient observed with the photometer differed somewhat from that determined with a spectrophotometer because of the large amount of stray radiation in the light source of the photometer. With sulfanilamide, for example, the "effective" ϵ was 10,100 liter mole^{-1} cm.$^{-1}$, whereas that determined with monochromatic radiation of 2537 Å. was 14,700. Nevertheless, this discrepancy has no significant effect on the order of magnitude of the sensitivity obtained.

The presence of stray light was also exhibited by the variation of the extinction coefficient with concentration. Since Beer's law was not applicable, it was necessary to construct a calibration chart of log I_0/I *vs.* concentration for each substance of interest.

Table I. Dependence of Sensitivity on Extinction Coefficient

Extinction Coefficient *Liter mole*$^{-1}$ *cm.*$^{-1}$	Minimum Detectable Concentration *Mole liter*$^{-1}$
10,000	3×10^{-8}
1,000	3×10^{-7}
100	3×10^{-6}
10	3×10^{-5}
1	3×10^{-4}

Applications

Figure 2 shows three typical calibration curves obtained with the ultraviolet photometer.

The sensitivity of the ultraviolet photometer toward phenol is probably comparable with that of any chemical method, for with a precision of 0.2 per cent in the light intensity it is possible to determine changes of approximately 3×10^{-5} gram of phenol per liter of water. This type of instrument may be very useful, therefore, in the analysis of phenol in water.

The limit of detection of sulfanilamide is even lower than that for phenol and is of the order of 2×10^{-6} gram per liter. A direct analysis of biological fluids is not possible, however, because of the presence of many other interfering substances which also absorb ultraviolet light. Nevertheless, the instrument will be very useful in a study of the adsorption and diffusion of sulfa compounds in various artificial media.

The results obtained with phenol and sulfanilamide are typical examples of the sensitivity one may expect with aromatic substances. Ultraviolet photometry would also be applicable to the analysis of organic substances such as ketones, conjugated hydrocarbons, heterocyclic compounds, and other ultraviolet-absorbing substances when they are dissolved in nonabsorbing solvents.

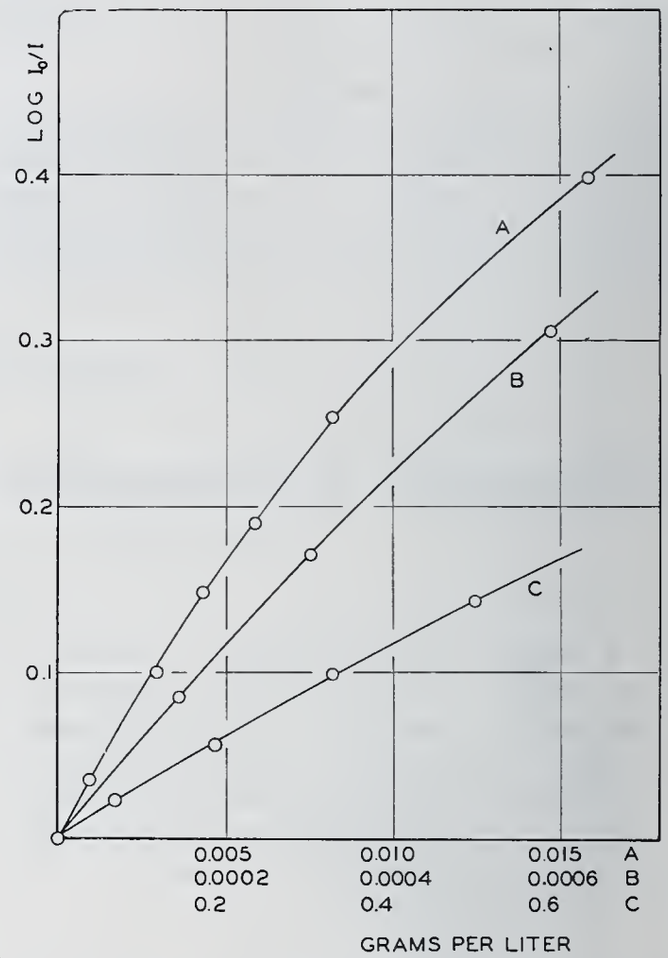

Figure 2. Calibration Curves
A. Phenol *B.* Sulfanilamide *C.* Potassium nitrate

An application to inorganic analysis, the calibration curve for potassium nitrate, is also illustrated in Figure 2. In this case the sensitivity, about 3×10^{-3} gram per liter, is not so high as that obtained with aromatic compounds, but the instrument would still afford a very rapid and convenient method for the analysis of nitrates.

These examples are only a few illustrations of the applications of this photometer. Many other involved and tedious chemical analyses, particularly of aromatic substances, can be replaced readily by ultraviolet photometry. To determine the sensitivity toward any particular substance, it is merely necessary to estimate the extinction coefficient from data usually available in the literature and to apply Equation 1 to calculate the minimum detectable concentration. A series of such estimates has been summarized in Table I to enable one to tell at a glance whether the desired sensitivity can be attained with a given compound.

Acknowledgment

The author is indebted to H. Campaigne, Chemistry Department mechanic, for advice and assistance in the construction of the carriage for the absorption cells.

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Determination of Specific Gravity of Dry Paint Pigments

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DIFFICULTY has been experienced in getting accurate, reproducible results in determining the specific gravity of dry paint pigments by the pycnometer bottle method when using oil, water, kerosene, or alcohol as a wetting medium (*1*). The errors encountered were: evaporation of liquid from glass joints of the pycnometer bottle, incomplete removal of surface-adsorbed air, and moisture condensation on the exterior of the pycnometer bottle. This procedure requires a very long treatment under vacuum, presents a hazard due to possible collapse of the desiccator under high vacuum, and is too complicated for routine tests for specific gravity of paint pigments.

Table I. Specific Gravity of Titanium Dioxide Pigments

Sample		Specific Gravity	Variation from Average	% Variation from Average
1		3.859	+0.007	0.18
		3.849	−0.003	0.08
		3.850	−0.002	0.05
		3.851	−0.001	0.03
		3.850	−0.002	0.05
		3.856	+0.004	0.10
		3.853	+0.001	0.03
	Av.	3.852	0.003	0.07
2		3.854	+0.001	0.03
		3.854	+0.001	0.03
		3.851	−0.002	0.05
		3.852	−0.001	0.03
		3.853	0.00	0.00
	Av.	3.853	0.001	0.03
3		3.851	−0.003	0.08
		3.856	+0.002	0.05
		3.853	−0.001	0.03
		3.858	+0.004	0.10
	Av.	3.854	0.0025	0.06

A rapid, accurate, reproducible method for the determination of specific gravity of such materials is presented here. Using heat and a high-speed centrifuge, pigments may be deaerated and wetted and their specific gravities determined rapidly with a minimum of equipment and skill, and with an accuracy of 5 parts per thousand.

Procedure

Fill a 600-ml. Berzelius beaker with kerosene oil. Prepare a suitable specific gravity tube by reducing a test tube 3.5 cm. in diameter to a length of 7 cm. At opposite sides of the tube and close to the top, punch two small holes through which a fine platinum or steel wire can be attached to act as a handle, so that the tube may be suspended from the hook above the analytical balance pan. Prepare a small glass stirring rod, slightly longer than the tube. Wash the tube and stirrer with cleaning solution followed by an alcohol wash, heat, and cool in a desiccator to remove moisture. Insert the stirrer in the tube, place in the 600-ml. beaker of kerosene, and bring the entire contents to 25° C. in a thermostat-controlled water bath. Wipe the kerosene from the suspending wire, support the beaker over the balance pan, and weigh the tube and stirrer completely submerged in kerosene at 25° C., after making certain that the temperature inside the test tube has had sufficient time to come to equilibrium with the surrounding temperature.

Remove the tube from the kerosene bath and weigh into it, by difference, from 1.5 to 25 grams of the paint pigment, depending on the specific gravity of the pigment, the pigment being previously dried at 105° C. for 2 hours. For pigments of low specific gravities, such as blacks and blues, weigh about 1.5 grams; for opaque white pigments, about 12 grams; for red lead, about 25 grams. Add sufficient kerosene to the pigment to wet it entirely and leave about 0.6 cm. (0.25 inch) of clear liquid above the pigment. Place the tube containing the pigment and stirrer in another beaker of kerosene and heat for 0.5 hour on a hot plate at a temperature of 65° to 70° C. with frequent stirring to remove air and other occluded matter and entirely wet the pigment. (This produces a small change in the specific gravity of kerosene and a negligible change in the final weight of the pigment in kerosene.)

Remove the tube from the kerosene bath, fill with kerosene to about 0.6 cm. (0.25 inch) from the top, and centrifuge at about 2000 r. p. m. for 0.5 hour. Remove the tube and carefully fill with kerosene. Place the tube and its contents again in the original 600-ml. beaker and place in the water bath at 25° C. When the temperature of the entire contents, inside and surrounding the tube, is 25° C., weigh the tube as before. The increase in weight represents the weight of the pigment in kerosene. The difference between the weight of the pigment in air and the weight in kerosene represents the weight of kerosene displaced. Accurately determine the specific gravity of the kerosene at 25° C. (It is not necessary to determine the specific gravity of kerosene after testing each of several pigments.) The specific gravity of the pigment is calculated from the formula:

$$\text{Specific gravity of pigment} = \frac{\text{weight of pigment in air}}{\text{weight of pigment in air-weight of pigment in kerosene}} \times \text{sp. gr. of kerosene}$$

Table II. Specific Gravity of Titanium-Calcium Pigment

Sample		Specific Gravity	Variation from Average	% Variation from Average
1		3.197	−0.002	0.07
		3.201	+0.002	0.07
	Av.	3.199	0.002	0.07
2		3.207	+0.0005	0.02
		3.206	−0.0005	0.02
	Av.	3.2065	0.0005	0.02

Discussion

The method outlined is direct and easily performed, and involves no detailed, difficult procedures. The errors of the pycnometer method have been eliminated since evaporation or moisture condensation is prevented by the fact that the apparatus is weighed both times submerged in the same liquid at the same temperature. Air and other occluded gases are removed more thoroughly by the double process of heating and centrifuging than by the single process of vacuum treatment. The accuracy of the results, as noted in Tables I and II, is 5 parts per thousand or better.

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THE views presented in this article are those of the writers and should not be construed as the official views of the Navy Department.

Laboratory Deodorizer for Fats and Oils

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STEAM deodorization is one of the fundamental processes in edible oil and fat technology and is practiced commercially on a very wide scale. It is, however, a relatively troublesome operation to conduct in the laboratory.

The operation involves blowing a heated batch of oil with steam under reduced pressure. Because the pressure required for the best operation is somewhat below the vapor pressure of water at ordinary temperatures, it is somewhat difficult to maintain vacuum upon the system. Multiple-stage steam ejectors such as are used commercially are not practicable for laboratory use. Mechanical oil-sealed vacuum pumps are not generally used because of the difficulties involved in completely condensing and trapping relatively large amounts of water vapor. Water ejectors operating on ice water or cooled brine are effective, but considerable auxiliary equipment is required for the constant delivery of large quantities of a cold liquid at high pressure.

During the deodorizing operation, steam must be delivered to the apparatus at a constant and easily controllable rate. The generation and delivery of the steam also present some difficulty and require the use of a steam generator of special and usually rather elaborate design.

The laboratory deodorizer described here is greatly simplified, in comparison with the conventional apparatus. It should be of particular interest to oil and fat laboratories which have only limited or occasional use for such equipment, and consequently have hesitated to devote space and funds to the usual deodorization setup. The entire apparatus, including the steam generator, is made of Pyrex, and may be fabricated by a glassblower of average ability. Vacuum for the apparatus is supplied by an ordinary rotary oil-sealed pump.

The distinctive feature of the apparatus, and the one which makes possible its simple design, is the relatively high

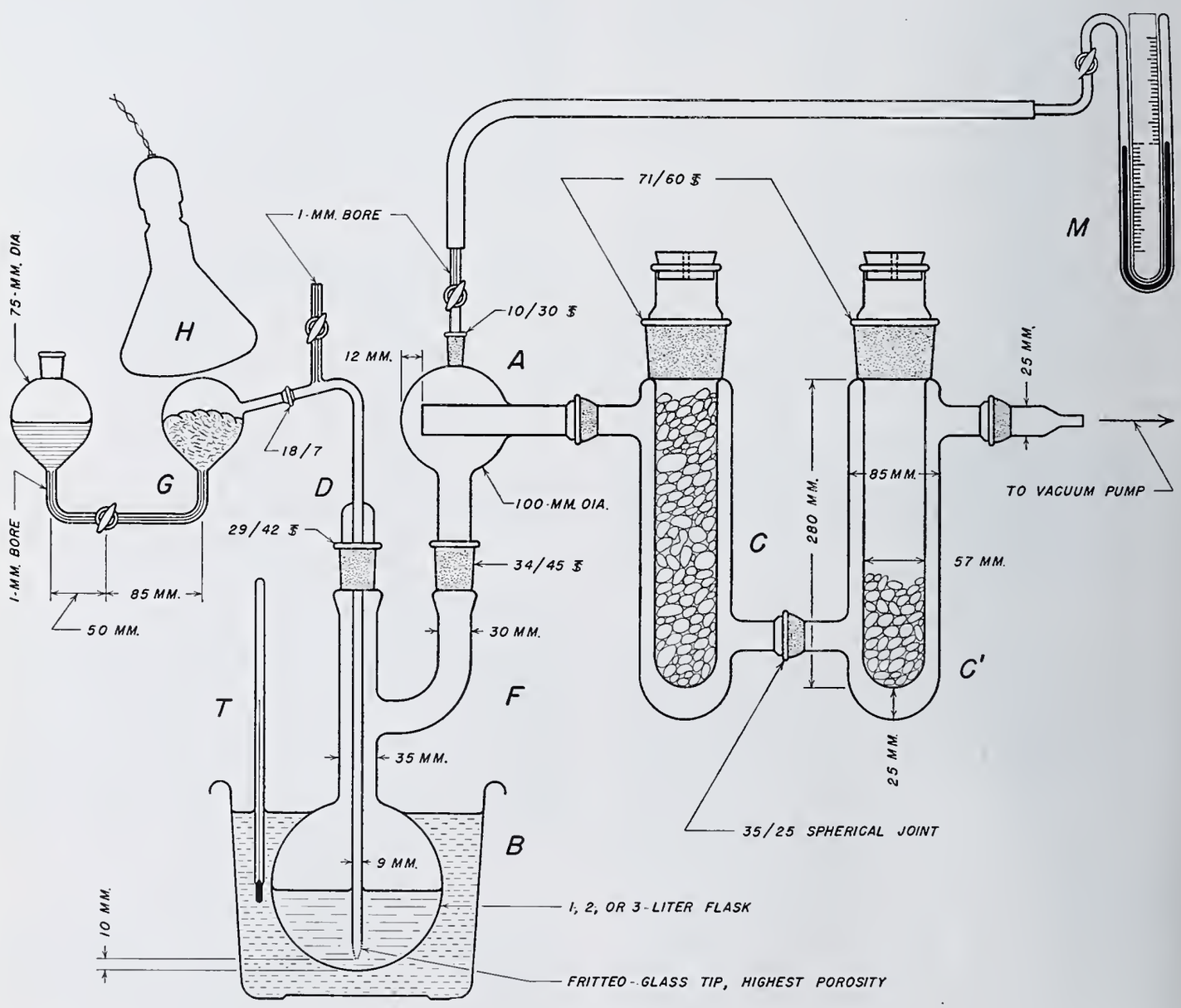

FIGURE 1. DIAGRAM OF APPARATUS

vacuum under which it operates—namely, about 1 mm. of mercury, instead of the usual 5 to 10 mm. under which most installations operate. Since steam requirements in deodorization are directly proportional to the total pressure (*1*), the amount of steam which must be generated and condensed is extremely small. This condition permits the use of a steam generator of novel design, and makes it feasible to condense all vapors in simple dry ice traps.

All volatile substances removed from the oil are quantitatively recovered in the condensers. The apparatus is useful for certain high-vacuum steam-distillation work other than deodorization and has been successfully used to reduce the free fatty acid content of crude vegetable oils, to strip out unreacted glycerol or free fatty acids after re-esterification reactions, and to separate degradation products from heat-treated oils.

The apparatus is illustrated in Figure 1. It is composed entirely of small Pyrex units equipped with standard ground-glass joints which permit the use of duplicate units in the original assembly. Spherical joints at certain points lend flexibility and make the apparatus easy to assemble. The ground joints may be lubricated with the oil or fat which is to be deodorized.

The steam generator, *G*, consists of two bulbs connected by capillary tubing. One bulb serves as a water reservoir. The other bulb is partially filled with asbestos, the top layer of which is covered with a film of carbon black, to enhance the absorption of heat. The carbon coating is conveniently applied to the asbestos by suspending a quantity of carbon in water and dropping the suspension on the asbestos surface from a pipet, while maintaining suction on the opposite end of the system. The rate at which water is admitted from the reservoir and at which steam is generated is easily controlled by the grooved stopcock between the two bulbs. Heat necessary to generate the steam by evaporation from the surface of the asbestos is supplied by a 250-watt infrared lamp, *H*. For critical work the water in the reservoir may be boiled to remove dissolved oxygen and then kept in an oxygen-free atmosphere by means of a hydrogen- or nitrogen-filled expansion bulb connected to the reservoir outlet. The steam delivery tube, *D*, is provided with a fritted-glass tip to distribute the steam flow.

The flask, *F*, which contains the oil to be deodorized, is fabricated from a Claisen-type distilling flask, and may be of any desired capacity within the range of about 1 to 3 liters. The flask is preferably heated by means of an oil bath, *B*, with the thermometer, *T*, indicating the temperature of the bath. A good deodorization rate is attained at temperatures of 425° to 475° F. (218° to 246° C.). If the apparatus is to be used for any purpose where it is important to know the exact temperature of the contents of the flask, a thermometer or thermocouple well should be inserted through a ground-glass joint directly into the body of the flask.

The spray trap, *A*, may be insulated or provided with an electrical resistance heater to prevent excessive reflux when stripping large quantities of fatty acids or other volatile constituents from the oil.

Pressure on the system is indicated by the manometer, *M*, which is connected to the spray trap by means of heavy rubber tubing. It is essential that all vapor passages be made large, as indicated in Figure 1, to avoid an appreciable pressure gradient through the apparatus.

Condensers *C* and *C′* are cooled with a dry ice–acetone mixture or with powdered dry ice. Single-hole rubber stoppers serve to keep warm air away from the interior of the condensers and permit the escape of gaseous carbon dioxide.

A single charge of dry ice will generally suffice for a deodorization. The dry ice is well insulated from the warm air of the room by the evacuated space surrounding the inner shell of each condenser. It will be found that practically all vapors from the flask will be retained in the first condenser. The second condenser functions largely as a safety device, to protect the pump in the case of accidental failure of the first condenser.

The proper sequence of operations in starting the deodorization operation is more or less obvious. After the apparatus is charged with oil, water, and dry ice, the vacuum pump is started, the heating lamp is turned on, the stopcock of the steam generator is gradually opened until the desired rate of steam generation is attained, and the oil bath is then heated to the operating temperature.

After deodorization is completed (usually 30 to 60 minutes), the hot oil bath is removed. The flask is then cooled by cold oil, a current of cool air, or other means, until the deodorized oil has reached a temperature of 100° to 150° F. (38° to 66° C.). The stopcocks leading to the manometer and the water reservoir are then closed, the manometer is disconnected, the vacuum pump is stopped, and vacuum on the system is broken by cautiously and simultaneously opening the stopcocks leading to *A* and *D*. This procedure avoids drawing oil back into the steam generator or blowing excessive quantities of air through the deodorized oil.

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Mayonnaise and Salad Dressing Yolk Content

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THE circulation in commerce of liquid and frozen egg yolk products varying in egg yolk solids content, and, more particularly, the recent promulgation of a definition and standard of identity for "egg yolk" by the Food and Drug Administration (*2*), call for a reconsideration of the analysis for "egg yolk" of such food products as mayonnaise and salad dressing.

The Association of Official Agricultural Chemists (*1*) gives an equation which expresses the per cent of yolk in the sample as a function of the per cent of nitrogen and the per cent of phosphorus pentoxide.

$$\% \text{ yolk} = 75.69\,P - 1.802\,N \qquad (1)$$

when P equals per cent of total phosphorus pentoxide and N equals per cent of total nitrogen. The per cent of egg yolk calculated from this equation is in terms of egg yolk having a total solids content of 50.5 per cent. Such a yolk can be prepared in the laboratory only and has been called "theoretical" yolk. Commercially produced egg yolk material contains various proportions of adhering egg white and has, therefore, a total solids content substantially below 50.5 per cent. The equation and other directions of the Association of Official Agricultural Chemists (*1*) do not give sufficient data to enable one to express the analytical results in terms of commercial yolk, and do not indicate that the percentage of yolk content calculated by the equation is the percentage of yolk of 50.5 per cent solids content.

How much egg white is admixed with the 50.5 per cent yolk in a commercial yolk depends on the definition of the commercial yolk; as defined by the provisions and regulations of the Federal Food, Drug and Cosmetic Act of 1938 (*2*), commercial yolk contains 43 per cent total solids. This 43 per cent commercial yolk can be considered to be a mixture of 80.42 parts of yolk of 50.5 per cent solids content and 19.58 parts of white of 12.2 per cent solids content.

In this discussion, as well as in the publications of the Association of Official Agricultural Chemists, it is assumed that all nitrogen and phosphorus pentoxide determined by an analysis of the mayonnaise or salad dressing are derived from egg yolk and egg white only (*3*).

Because of the need for determining the content of commercial yolk in salad dressing or other food products, the

following equations are presented. The numerical basis of the equations is the following analytical data taken from a paper by Mitchell (*4*):

	Total N %	Total P_2O_5 %
In theoretical yolk (50.5% solids content)	2.61	1.38
In egg white (12.2% solids content)	1.72	0.04

The same analytical fundamental data are the bases for the equations of the Association of Official Agricultural Chemists (*1*).

The equation which expresses the analytical results as per cent of egg yolk of 43 per cent solids in the sample is

$$\%\ \text{yolk}_{(43)} = 94.26\ P - 2.192\ N \qquad (2)$$

when P is the per cent of phosphorus pentoxide and N the per cent of nitrogen present in the sample. The per cent of egg white of 12.2 per cent solid content present in the sample outside of the egg white present in the commercial yolk of 43 per cent solid content is

$$\%\ \text{white} = 61.24\ N - 133.48\ P$$

when P is the per cent of phosphorus pentoxide and N the per cent of nitrogen present in the sample.

The equation which gives the per cent of commercial yolk of any (g per cent) solids content in the sample is

$$\%\ \text{yolk}_{(g)} = \frac{2899\ P}{g - 12.2} - \frac{69\ N}{g - 12.2} \qquad (3)$$

The relation of this equation to the equation for 50.5 per cent yolk content

$$\%\ \text{yolk}_{(50.5)} = 75.69\ P - 1.802\ N \qquad (4)$$

can be seen by writing Equation 3 as follows:

$$\%\ \text{yolk}_{(g)} = (75.69\ P - 1.802\ N)\ \frac{50.5 - 12.2}{g - 12.2} \qquad (5)$$

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Ground Glass for Spot Testing in Solvent Extractions

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ABOUT five or six years ago the writer noted the ease with which a ground-glass plate, such as is used in photography, becomes soiled in handling. Application of this observation led to a simple spot test for determining the completeness of extraction of fatty oils, or other nonvolatile liquids, with volatile solvents.

The test depends on the transparency which an oil or other nonvolatile liquid produces on ground glass, and is analogous to the familiar transparency which an oil produces in paper. The most plausible explanation is, briefly, that the oil film, by its refractive power, suppresses both the reflection of the incident light rays and the spreading of the emergent refracted rays, causing the latter to assume more nearly parallel courses, such as would have occurred had they passed through plate glass with polished surfaces. This hypothesis predicts maximum transparency when the liquid and glass have the same refractive index, a condition which is approached in the case of most fatty oils and ordinary ground glass of the grade commonly used for photographic focusing.

PROCEDURE. Drops of the solution under test and of the solvent are placed on a clean ground-glass surface and allowed to evaporate. Depending on the amount of solute present, the transparencies formed vary from that of the clean plate with the pure solvent to a disk of clear transparency of about the same area as that of the spot before evaporation. Between these extremes lies a wide variety of intensities, and at low concentrations of solute one or more circular lines of approximately hairline thickness are formed. The residue can also be viewed against a dark background, when it will appear dark instead of as a clear transparency. When the solvent is impure, the blank test may show a slight hairline transparency of varying intensity, depending on the impurity in the solvent, and this should be taken into consideration when making a spot test on an extraction.

Sensitivity

As described, the limit of sensitivity of the test is about one part of solute in 20,000 parts of solvent (0.005 per cent). Greater sensitivity can be obtained by allowing two or more drops of solution to evaporate on the same spot, or by dusting the spots with a dark powder, such as a fine carbon bleach, and shaking off the excess. This procedure brings out the residual solute in sharp relief, as in fingerprint detection. A magnifying glass frequently can be used to advantage, but for most purposes it will suffice to examine, with the unaided eye, the transparency produced by a single drop of solution.

Applicability

Though originally devised for, and used on, solutions of fatty oils in petroleum ether, ethyl ether, etc., this test may be applied to most solutions of nonvolatile liquids in volatile solvents, such as glycerol and sulfuric acid in water solution. In these cases the test is not quite so rapid as in the case of the more volatile solvents, and may be hastened by the gentle application of heat in the early stages of the evaporation. Much care must be taken, however, near the end of the evaporation to avoid loss of glycerol or sulfuric acid, both of which are somewhat volatile at relatively moderate temperatures.

This test should be found useful in gaging the extraction of oils and fats, glycerol, and other substances. A typical application is its use as an index to completeness of extraction with petroleum ether in the determination of unsaponifiable matter.

Precautions

The solution tested must be free from nonvolatile material other than that which is to be detected or estimated, regardless of whether such extraneous nonvolatile matter produces a transparency or an opacity. Salts and similar solids produce opacities, and if present in sufficient quantity, obscure the test. Fortunately, such substances are rarely extracted by the usual solvents employed in the extraction of fats and oils.

A Mercury-Balance Pressure Regulator

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OF THE numerous pressure regulator designs reported, the large majority involve elaborate and costly apparatus which permits neither rapid assembly and easy adjustment nor portability and application to different operations involving reduced pressure.

Broadly, pressure regulators can be classified as intermittent or continuous in operation. Most of the elaborate electrical assemblies maintain the pressure at an average value by intermittent pumping, or intermittent leaking with constant pumping. Basically, all these intermittent devices would appear to produce pressures which oscillate above and below the average value, the degree of oscillation depending upon the ballasting effect of the entire system and the sensitivity of the compensating device. Continuous regulators, on the other hand, adjust the amount of leakage so that the sum of gas from the vacuum system plus the controlled leakage equals the capacity of the pump. The precision of pressure regulation then depends on the precision of the proportioning valve—i. e., the sensitivity of the regulator—which is a compound factor determined by the smallest change which causes compensation, the rapidity of compensation, and the magnification of the compensation. Ideally, a continuous regulator should react swiftly and to an exaggerated degree to a minute departure from established pressure.

The designs involving direct control of leakage by mercury balance appeared to offer a precise method of obtaining continuous regulation with all three of these characteristics. The precise types devised by Schierholtz (*4*) and Thelin (*6*) used the translation of mercury in a manometer to actuate a lever which controlled the magnitude of the leak. The mercury control of Emerson (*3*) employed mercury entrained in the stream of leakage to obtain the final degree of throttling. The designs of Swayze (*5*) and Watts, Riddick, and Shea (*7*) are examples of intermittent operation of pump and leak, respectively. The apparatus of Caldwell and Barham (*2*) employed a glass valve operated directly by the mercury in a manometer. Numerous other devices, including many of considerable complexity, have been reported.

Apparatus

Apparatus patterned originally after those of Schierholtz and Thelin was improved through four successive models. The use of a mercury column to balance the excess pumpage by leakage was found to be precise, rapidly responsive to disturbance of equilibrium, and capable of indefinite magnification of compensation.

Two modifications of the final device are presented here. They employ the same principle as those of Schierholtz and Thelin, but have the mechanical beam eliminated and incorporated in the manometer; one has a loop type of manometer which permits wide range and easy adjustment. Both require only elementary glassblowing technique to construct. The construction is shown in Figures 1 and 2.

Three sizes of tubing were used: 1-mm. capillary, 8 mm. in outside diameter, and 19 mm. in outside diameter, as indicated by *A*, *B*, and *C*, respectively, in Figures 1 and 2 (lettered identically). The glass manometer was supported in two places: by the spring, *D*, and the pivot, *E*. The spring contained 90 turns of 26-gage piano wire coiled on a 4-mm. rod and was connected by a swivel, *F*, to the thumbscrew, *G*, which adjusted the tension in the spring. Pivot *E* was a glass tube at right angles to the manometer, which served both as the vacuum connection and the shaft about which the manometer rotated. The thumbscrew and pivot were supported by the cold-rolled iron rod, *H*, which was attached to a ring stand by a right-angle clamp. To this rod was attached a rubber stopper, *I*, which served as the valve seat. The stopper was preboiled in dilute alkali and the glass seat was ground flat with a fine pocket whetstone.

Operation

The regulator in Figure 1 was operated by connecting to the vacuum system with the spring relaxed. When evacuated to the proper degree, the spring was tightened until the valve barely opened. While the actual opening was not usually easily visible, the effect was noted on a separate manometer. Once adjusted, a decrease in pressure in the system caused the mercury in the manometer to flow into the reservoir, unweighting the manometer-beam and opening the valve. An increase in pressure caused opposite effects.

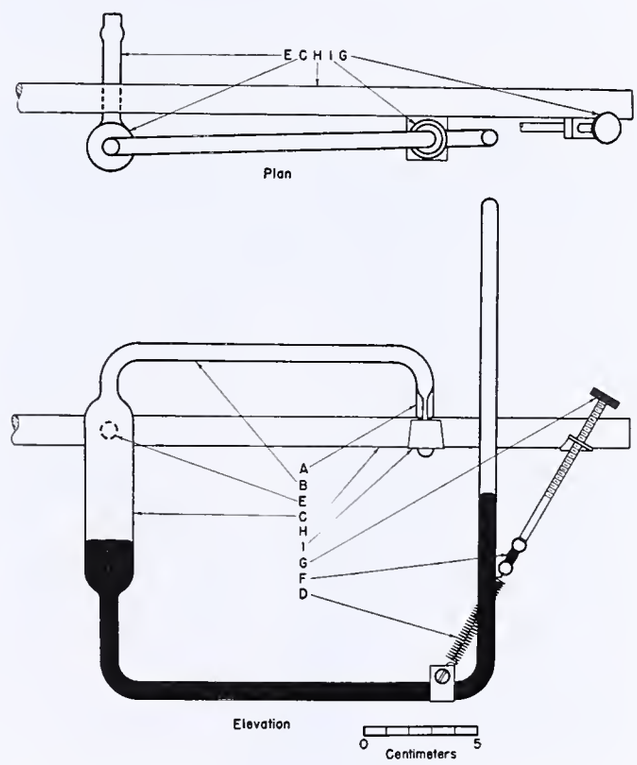

FIGURE 1. PRESSURE REGULATOR WITH CLOSED MERCURY MANOMETER

The regulator in Figure 2 was similar in operation, except that the stopcock was left in the open position while the proper degree of vacuum was established roughly by adjusting the spring tension. The stopcock was closed and the final adjustment made by adjusting the spring tension. A decrease in pressure then caused the mercury to flow out of the closed end of the manometer, unweighting the manometer-beam and allowing the spring tension to open the valve.

Precision of Regulation

The precision obtained with the regulator shown in Figure 1 was about 0.1 mm. of mercury; that of the type in Figure 2 was not readable with the naked eye. Many factors influence precision. The precision obtained with these regulators was the algebraic sum of these variables under given conditions and was by no means the ultimate precision of which the device was capable.

Spring tension affected sensitivity greatly. A spring having a small load-deflection ratio was more sensitive than

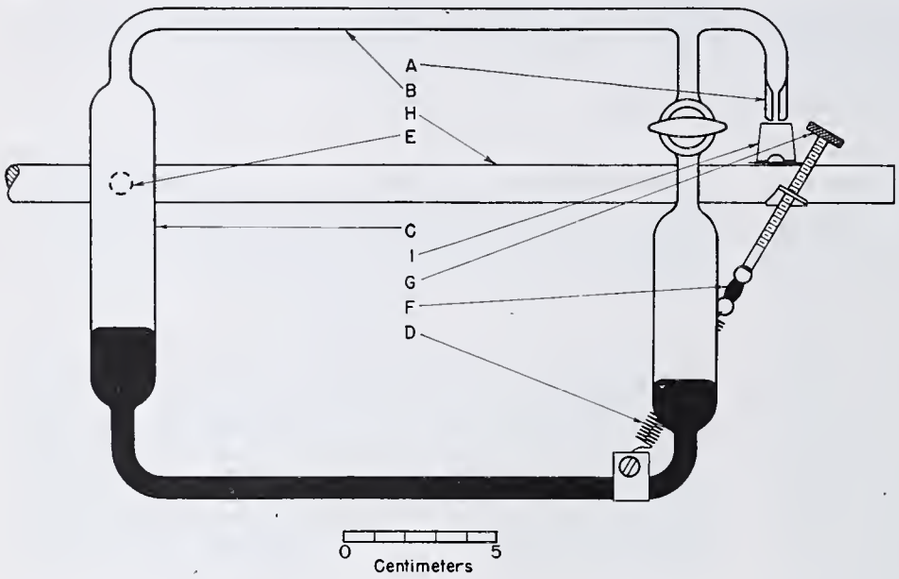

FIGURE 2. PRESSURE REGULATOR WITH LOOP TYPE OF MANOMETER

a stiffer spring. The diameter of the tubing in the closed limb of the manometer controlled the weight of mercury shifted for a given pressure change, hence larger tubing in the closed limb of the manometer greatly increased sensitivity; because of location the size of the open limb was unimportant. A bubble of air was always trapped in the closed end of the manometer in the Figure 2 type of regulator; a larger bubble resulted in increased sensitivity. A bubble trapped above the mercury in the Figure 1 type of regulator increased the sensitivity and increased the pressure range over which the device was operative. While the pivot bearing on these regulators was the glass tube, better practice would be to sheath this tube with a machined, split metal bushing, since glass tubing is rarely round and is apt to stick in the bearing. It would likewise be better practice to connect to the vacuum connection, *E*, with a loop of thin-walled rubber tubing, prevented from collapsing by Raschig rings inserted at intervals, and with the other end of the loop fastened permanently to the support bar, *H*, so that torque on the regulator by changing the position of the suction tubing would not change the adjustment of the regulator.

Both regulators were independent of variations in atmospheric pressure. Adjustment to a predetermined pressure by means of the thumbscrew, *G*, having 32 threads per inch, was easily effected. Both regulators controlled pressure with uniform facility over the entire pressure range. Both regulators were susceptible to "bouncing" under certain conditions—i. e., an oscillation of the mercury column and concomitant movement of the manometer-beam, causing intermittent admission of air. It was found to be due to parallelism of valve seat and face, and was eliminated by an imperceptible bending of the valve seat support, so that valve seat and face were not quite parallel and hence the valve could not close completely. The bouncing apparently was due to complete closure of the valve. In order to open the valve, pushing against atmospheric pressure required overcompensation of the mercury-balance, and then rapid overcompensation in the opposite direction resulted owing to the sensitivity of the device, again causing sealing of the valve. This bouncing phenomenon has been noted in other regulating devices (*1*) using a mercury column and is due to similar causes.

Generally speaking, the Figure 2 type of regulator was more flexible and sensitive, but no stopcock under vacuum can be considered completely dependable under long periods of operation. Either regulator may be made capable of increased sensitivity by increasing the diameter of the tubing in the closed manometer limb, lengthening the beam, using a more elastic spring, or trapping a larger volume of air in the closed end of the manometer. Where great sensitivity is not required, a rubber cushion can be used to replace the spring suspension, but all attempts to use rubber have necessitated continual adjustment, owing to the fatigue of the rubber cushion.

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Electrolytic Preparation of Quinhydrone

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AN ELECTROLYTE used extensively in pH measurements is quinhydrone, which consists of bright platinum immersed in a saturated solution of quinhydrone. In view of the fact that quinhydrone is an equimolecular compound of hydroquinone and quinone, it was thought possible that hydroquinone, a relatively cheap product used in photography, might be oxidized to quinone by the use of an electric current. The resulting product would be free of foreign materials.

The apparatus consists of an outer cup of nonporous material 20 cm. (8 inches) high and 20 cm. (8 inches) in diameter and an inner cup of porous material 17.5 cm. (7 inches) high and 7.5 cm. (3 inches) in diameter. A carbon electrode, obtained from a dry battery such as those used in residential doorbell circuits, is placed in the outer cup (anode) and another is placed in the inner cup (cathode).

TABLE I. PREPARATION OF QUINHYDRONE

Expt. No.	Electrolysis Time *Hours*	Amperage *Amperes*	Hydroquinone *Grams*	Yield %
1	4.82	1	10	72.6
2	2.41	2	10	74.2
3	1.61	3	10	74.8
4	1.21	4	10	75.3
5	0.96	5	10	74.1

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The hydroquinone has an equivalence of 2; therefore one mole of hydroquinone would take 2 faradays for complete oxidation. As only half the quantity of hydroquinone used is to be oxidized, half of the required faradays for complete oxidation should be used, or one faraday per mole of hydroquinone.

The hydroquinone (20 grams) was dissolved in a suitable amount of water and poured into the outer cup of the electrolytic apparatus. Two to 3 grams of sodium sulfate were added and the level of the liquid was raised to within 2.5 or 5 cm. (1 or 2 inches) of the top of the cup. The inner cup was half filled with distilled water, 5 ml. of glacial acetic acid were added, and the cup was filled with distilled water to within 1.9 cm. (0.75 inch) of the top. The current was then turned on at various amperages and for various times (Table I). Sodium sulfate was added to the outer cup liquor in small amounts from time to time to maintain a constant amperage. At the completion of the required oxidation period, the solution in the inner cup was concentrated and cooled, and the quinhydrone was filtered off. The yield was about 75 per cent of the theoretical.

The purity of the quinhydrone in each experiment was 98 per cent or better in each case. In the opinion of the author a higher yield may be obtained by using the mother liquor repeatedly.

Accurate Low-Pressure Gage

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LOW-PRESSURE gages commonly found in physics and chemistry laboratories are either of the Bennert closed U-type, or one of the many modifications of the McLeod type. The Bennert type is satisfactory for pressures above 1 cm. of mercury, but is not very accurate for lower pressures. For the low-pressure range the McLeod gage is usually used, but its application is limited by the fact that it depends on increasing the pressure in one part of the system. This increase in pressure will, in the case of vapors from liquids having high boiling points, lead to a condensation of the vapors, which will make the measurements useless. Thus in the case of measuring the vapor pressure of water of crystalline hydrates, the pressure cannot be increased above that over pure water at the same temperature without bringing about dew formation.

A number of methods have been used for increasing the accuracy of such measurements, one of which involves the replacement of mercury by a liquid of low density and negligible vapor pressure, always being certain that the vapors being measured are not soluble in the confining liquid.

The floating tube barometer described by Caswell (*1*) in 1704 and later adapted to use in what has been called the steelyard barometer (*3*) embodies the basic principles of a recent instrument known as the Dubrovin (*2*) gage, which may be purchased from scientific apparatus houses. Since there appears to be no published description of the theoretical background of the floating barometer, it seemed worth while to develop the equation for the magnification factor, and to give directions for making a gage which would be useful in vacuum distillation work, as well as around physical chemistry and physics laboratories.

Figure 1 shows an inverted closed cylinder floating freely over mercury. Its lateral motion is controlled by three point guides at top and bottom lightly touching the inside walls of the containing vessel. The device is placed on its side and a high vacuum is created by means of a good oil or diffusion pump. This removes all the air and adsorbed gases from the inside and outside of the floating tube. It is then set up on end and atmospheric pressure is slowly restored. If the density of the tube material is less than that of mercury, the tube will float and the inside of the floating tube will be entirely filled with mercury. Now if the external pressure is gradually reduced, the tube remains stationary until the external pressure becomes equal to the height of the mercury column inside the tube above the outside level. (Capillary forces are for the moment neglected.)

Reducing the pressure still further results in the condition shown in the figure. The height, N, measures the pressure in the large cylinder. As the pressure is progressively reduced, the mercury column drops while the tube itself rises. Thus a drop of 1-cm. pressure results in a drop of 1 cm. in height N, while H may increase 10 or 20 cm., corresponding to multiplying factors of 10 or 20.

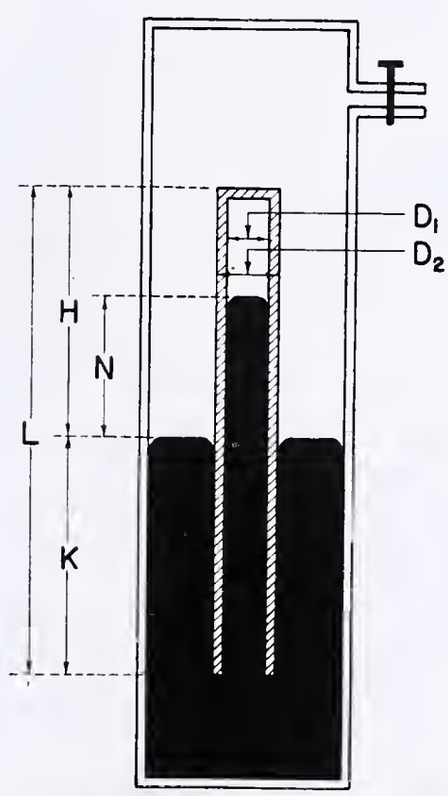

FIGURE 1

Regarding the tube as a freely floating body at equilibrium, constrained by guides to move only in a vertical direction, and assuming the gas pressure inside the floating tube to be zero, we may equate the sum of the downward forces to the sum of the upward forces. Height N of the liquid inside the tube depends only on the external pressure, P.

The pressure in the liquid at the base of the floating tube is made up of the sum of the gas pressure, $P = Ng\rho$, and the pressure of the liquid column, K, which is $Kg\rho = (L - H)g\rho$, in which g and ρ represent the force of gravity and the density of the liquid. This total pressure $g\rho(N + L - H)$ over the annular ring area of

the tube $\frac{\pi}{4}(D_2^2 - D_1^2)$ exerts an upward force $\frac{\pi g\rho}{4}(D_2^2 - D_1^2)(N + L - H)$. To this we add F, the algebraic sum of all other constant forces exerted by surface tension, floats, etc. Hence:

$$\text{Total upward force} = \frac{\pi g\rho}{4}(D_2^2 - D_1^2)(N + L - H) + F$$

The downward force is made up of W, the weight of the tube, and the pressure, $P = Ng\rho$, over the sectional area of the tube $\frac{\pi}{4}D_2^2$:

$$\text{Total downward force} = \frac{\pi N g\rho D_2^2}{4} + W$$

For such a body in equilibrium we may equate the upward to the downward forces and obtain

$$\frac{\pi g\rho}{4}(D_2^2 - D_1^2)(N + L - H) + F = \frac{\pi N g\rho D_2^2}{4} + W$$

Simplifying and grouping the constant terms as C, this becomes

$$-H = \frac{ND_1^2}{D_2^2 - D_1^2} + C$$

Differentiating, we obtain

$$-\frac{dH}{dN} = \frac{D_1^2}{D_2^2 - D_1^2}$$

This equation states that as N increases, H decreases and vice versa. It also states that the rate of change of H with respect to N is constant, since it depends only on the constant tube dimensions. The numerical value of this rate of change for a given instrument with constant tube diameter and constant wall thickness is accordingly the same for infinitesimal and for finite changes in H and N. Since the weight of the tube, the density of the liquid, surface tension forces, etc., included in the constant term, C, do not appear in the final equation, the rate of change of H with respect to N is independent of all such constant terms.

The absolute value of N depends on the pressure, P, in the outer tube and on capillary forces. The absolute value of H depends on the densities of the tube and the liquid, pressure P, and surface forces. In view of these facts it is evident that the ratio of the absolute value of H to N is meaningless. On the other hand, a decrease in the pressure, P, of 1 mm. of mercury is accompanied by a decrease in N of 1 mm. if the confining liquid is mercury. If simultaneously the tube rises 10 mm., then H has increased by 10 mm. and the change of H with respect to N is 10. This is then the true value of the multiplying factor or magnification. As indicated in the equation for the rate of change of H with respect to N, it can be calculated from the internal and external diameter of the floating tube, and is independent of all other factors. Both theory and experiment show that as the liquid inside the tube falls and the tube rises, the level of the outside liquid does not change. This means that the volume of the cylinder that comes out of the liquid equals the volume of the liquid that drops into the body of the fluid.

The instrument has the very distinct advantage over other mechanical and optical magnifying devices of not magnifying the error, if any, due to capillarity, adhesion to the tube, etc.

The apparent density of the tube may be changed either by making the lower submerged part of tube itself of a double-walled sealed cylinder or by attaching a separate float made of glass at its base. In this way even a platinum tube can be made to have the absolute value of H greater than the barometric height of 76 cm. of mercury, using mercury as the confining liquid. We then have a direct-reading magnifying barometer.

The guides serving to hold the tube erect make only slight contact with the outer tube, some clearance being allowed. When a reading is to be made, a light tap on the side of the outer tube momentarily releases contact, permitting the floating tube to rise or fall freely about its equilibrium position, which is easily reproducible to less than 0.2 mm. If we have a tube with a magnification factor of 20, a barometric change of 1 mm. of mercury is accompanied by a 20-mm. change of H. An error of 0.2 mm. in reading the height of H is an error of 1 per cent. Stated another way, a 0.01-mm. change in barometric pressure is the minimum change which can be observed directly without the use of a vernier. Since the accuracy of reading is independent of H, 10^{-2} mm. of mercury is the lowest pressure that can be read on an instrument whose magnification factor is 20 when the liquid used is mercury.

If in place of mercury we use Apiezon B oil, whose density at 25° C. is 0.861 gram per cc., the accuracy is greatly increased. With the above instrument having a magnifying factor of 20, and a minimum observable pressure change of 0.01 mm., the minimum observable pressure change becomes 0.01/15.7 = 0.00064 mm. of mercury, in which 15.7 is the ratio of the densities of mercury and Apiezon B oil at 25°.

The above considerations show that for mercury as a confining liquid the useful range of the gage lies between 10^{-2} and 10^3 mm. of mercury, whereas with Apiezon B oil the lower range is extended to 6.4×10^{-4} mm. A relatively high accuracy is achieved over a very large pressure range without the use of a vernier. Readings are made directly and are instantly observable. No operation such as changing volumes as in the case of the McLeod gage is necessary.

Mercury as a confining liquid has the great advantage of dissolving very few vapors of either organic or inorganic substances. It has the disadvantage of high density and accompanying inaccuracy in reading small pressure changes. It is this latter difficulty which the floating tube overcomes.

A concrete illustration of its use in the authors' laboratory of physical chemistry: In the determination of gas densities by direct weighing, the student is instructed to evacuate the bulb to 0.1 mm. or less and then weigh. This involves the use of a gage of the McLeod type. However, by including the floating gage in the system the pressure can be read directly.

Although the equation enables the calculation of the dimensions of the tube for any specific multiplying factor, the following have been found practical.

D_1	D_2	Wall Thickness	L	Magnification
Inch	*Inch*	*Inch*	*Inches*	
0.1225	0.1250	0.00125	10	24.25
0.3576	0.375	0.0087	12	10.03

Stainless steel, nickel, platinum, and glass tubes may be used with mercury. Since a uniform bore and wall thickness are essential to a uniform pressure scale and the thin-walled tubes which are necessary are stronger when made of metal, metal tubing is especially satisfactory. Strength is often necessary, since a rapid restoration of pressure in the outside container may cause the resulting mercury hammer effect to break out the top of the floating tube. To obviate this, the lower part of the tube may be made with a rather small opening, and thus avoid a rapid entry of the mercury.

Acknowledgment

The authors wish to thank the Superior Tube Company, Norristown, Penna., and the Summerill Tubing Company, Bridgeport, Penna., for their kindness in making up special tube sizes for use in the above studies.

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Automatic Apparatus for Determination of Small Concentrations of Sulfur Dioxide in Air

Application to Hydrogen Sulfide, Mercaptan, and Other Sulfur and Chlorine Compounds

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MANY units of the automatic apparatus described in earlier papers (*6*, *7*, *8*) have been in continuous operation during the last twelve years and they have provided complete records of sulfur dioxide concentrations in the atmosphere. The machines have required very little attention for servicing, and have given long periods of satisfactory operation.

Three years ago the authors experimented with a multiple-port stopcock-type metal valve to replace the numerous cam-operated poppet valves, and this innovation has given complete satisfaction, resulting in simple construction and trouble-free operation. Making use of this new valve, the authors have constructed an autometer which determines the sulfur dioxide (plus sulfur trioxide) and also independently the total volatile sulfur content of the atmosphere. The latter may also include hydrogen sulfide, carbon disulfide, mercaptan, and even substances like thiophenol or thiophene. Preliminary observations have shown that this apparatus is also applicable to volatile chlorine compounds like chloroform, carbon tetrachloride, ethylene chloride, and chlorobenzene, all of which can produce hydrochloric acid that is absorbed as readily as sulfur dioxide, and affects the conductivity like sulfuric acid.

Construction of Apparatus

The multiple-port stopcock, as used on the combined sulfur dioxide–total sulfur machine, is shown in Figure 1. For sulfur dioxide alone planes 3, 5, and 2 of the ports on plane 6 may be eliminated and the valve made correspondingly smaller. Stainless steel and bronze, or bronze and brass, have been used in its construction. The body and core are machined, then lapped with suitable steel blanks before lapping them carefully together to produce a smooth close fit. Finally the ports and grooves are made without disturbing the fit.

The valve is mounted in a vertical position on the support, *C*. It is operated, using a heavy stopcock grease and adjusting the collar, *B*, so that a minimum clearance is maintained to prevent the surfaces from binding. Adequate pressure is given by the cap and spring, *A*. The core is turned from below by a reduction gear system with which it is connected by means of a universal drive pin, *D*, that allows the core to remain centered in the valve body. A well-made valve of this type will run for many months without any attention.

Figure 2 is a diagram of the assembly. The valve, reduction gear system, pump, and motor are mounted on a bed plate. The absorbers are placed on a light-iron framework so that they will drain by gravity through the lowest ports of the stopcock. On an upper shelf is a 20-liter bottle containing the absorbing solution, and at a level between the supply bottle and absorbers are placed the pipets. Thus the solution passes through the valve three times by gravity: first into the pipets, then into the absorbers, and finally to waste. Accessory apparatus is the same as that described earlier (*6*, *7*).

Application

It has been known for a long time that appreciable amounts of hydrogen sulfide would pass through the sulfur dioxide absorber without producing a measurable effect on the conductance of the absorbing solution. The same has now been found to be true of the other sulfur and chlorine compounds mentioned above. Accordingly, if these compounds could be quantitatively oxidized or pyrolyzed to sulfur dioxide or

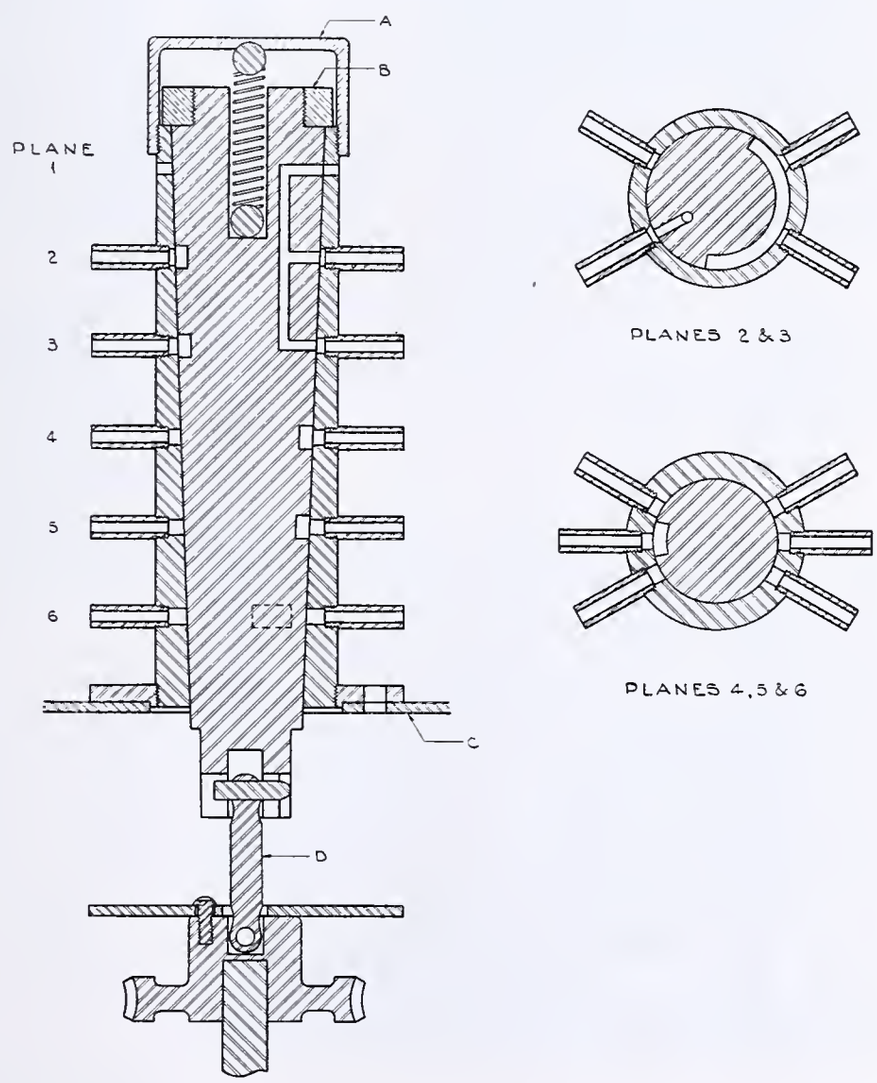

FIGURE 1. MULTIPLE PORT STOPCOCK-TYPE METAL VALVE, FOR CONTROLLING FLOW OF GASES AND LIQUIDS

hydrochloric acid, they could be determined with the same precision and sensitivity that characterize the sulfur dioxide analysis, for which the limit of detection is about 2 parts per billion.

The application of the sulfur dioxide autometer to the determination of hydrogen sulfide has long been envisioned, but until recently only a few preliminary experiments had been carried out.

In 1939–40 Philip Drinker borrowed an autometer from this laboratory and used it to determine carbon disulfide and hydrogen sulfide in the Lewistown Plant of the American Viscose Corporation. The results of this study were described as satisfactory by Reece, White, and Drinker (*4*). The carbon disulfide–air and hydrogen sulfide–air mixtures "were passed over heated platinum foil and the sulfur completely oxidized to sulfur dioxide." Evidence of completeness of oxidation was not presented, but this probably was not important because the authors state, "The instrument must be calibrated against known concentrations of sulfur dioxide, furnished, in our case, by the oxidation of carbon disulfide." The sampling rate was 5.5 liters per minute. The authors have been unable to attain complete oxidation of hydrogen sulfide at 15 liters per minute in a silica tube containing platinum foil and heated externally in an electric tube furnace.

Analysis of chlorinated hydrocarbons has been accomplished by several investigators using combustion methods.

Olsen, Smyth, Ferguson, and Scheflan (*3*) passed the vapors of carbon tetrachloride in air together with some water vapor, through a silica tube at 1000° to 1100° C., and determined the liberated hydrochloric acid by titration. They suggested the possibility of an automatic method based on conductivity, but did not try it out. Their procedure has been modified by Tebbins (*5*) who used a platinum catalyst in the combustion tube and reduced the temperature to 850° C. Other minor modifications have been made by Dudley (*1*), who employed a sampling rate of 2.5 liters per minute, as compared with a rate of 1 liter per minute employed by the earlier investigators.

The new sulfur dioxide–total sulfur autometer is really a double sulfur dioxide analyzer.

It has four identical absorbers, two of which are used for sulfur dioxide exactly as in the simpler machine. The other two receive an equal volume of air in a parallel line from the same source. To secure continuous operation, one pair of absorbers is aspirated while the other pair is recharged with solution. The second air stream is passed through a well-insulated 25-mm. silica tube in which a platinum wire, spirally wound and electrically heated, serves to oxidize other sulfur compounds to sulfur dioxide. The reaction is partly a pyrolysis, since carbonaceous material gradually deposits beyond the heater. The use of the internally heated tube results in a great saving of power with the large air flow employed (15 liters per minute) as compared with an externally heated tube, and also gives a quantitative oxidation. With smaller rates of air flow still less power would be required.

The hot end of the silica tube is connected to a Pyrex adapter by a ground joint which is maintained by holding tube and adapter together with a spring. The adapter tube is partly water-cooled, but extreme cooling of the gas is avoided, since there is some tendency to produce sulfuric acid in the heater, and this

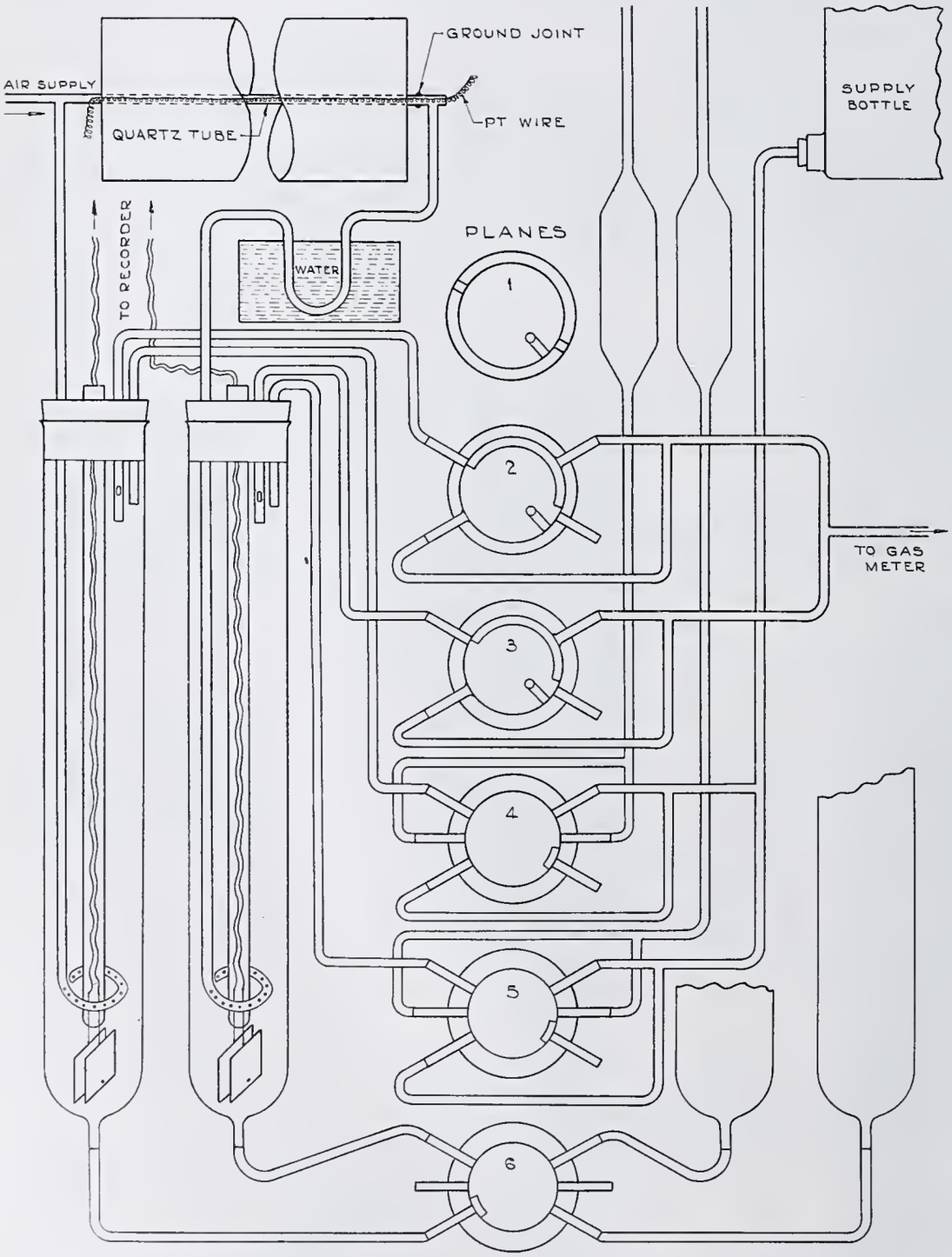

Figure 2. Diagram of Assembly for Simultaneous Determination of Sulfur Dioxide and Total Sulfur in Air

Table I. Determination of Sulfur and Chlorine Compounds

Compound	No. of Analyses	Energy	Gas Temperature	Element Found	Element Calculated	Compound Calculated	Recovered
		Watts	*° C.*	*Mg. S or Cl per 1000 l.*		*P. p. m. vol.*	*%*
Hydrogen sulfide	1	100	390	0.51	0.78	0.68	65
	1	100	390	0.98	1.37	1.19	71
	1	108	395	1.17	1.82	1.58	64
	1	108	395	1.97	3.30	2.87	60
	1	160	470	0.035	0.034	0.030	103
	2	160	470	0.080	0.075	0.065	107
	1	160	470	0.77	0.88	0.076	88
	1	160	470	0.91	1.07	0.93	85
	2	240	555	0.135	0.130	0.11	104
	6	255	570	0.67	0.70	0.61	96
	3	255	570	1.07	1.10	0.96	97
	1	258	573	2.22	2.36	2.05	94
	2	260	575	3.58	3.60	3.14	99
	2	366	650	0.20	0.195	0.170	103
	7	470	715	0.180	0.179	0.156	100
	6	470	715	0.74	0.75	0.65	99
	1	490	725	3.76	3.82	3.33	98
Carbon disulfide	1	250	565	1.69	1.68	0.73	101
	1	250	565	1.73	1.77	0.77	98
Ethyl mercaptan (C_2H_5SH)	1	250	565	0.050	0.049	0.043	102
	1	250	565	0.085	0.085	0.075	100
	1	250	565	0.72	0.70	0.61	103
	1	250	565	2.55	2.54	2.21	100
	1	250	565	3.59	3.61	3.24	99
Thiophenol (C_6H_5SH)	1	240	555	0.65	0.69	0.60	94
	1	240	555	1.08	1.08	0.94	100
Thiophene (C_5H_4S)	1	250	565	0.175	0.173	0.15	101
	1	265	575	1.95	1.79	1.56	109
	1	265	575	2.87	2.81	2.25	102
	1	250	565	5.90	6.25	5.45	95
	1	375	660	3.65	3.68	3.20	99
Chloroform	1	240	555	0.11	0.31	0.041	35
	1	235	550	0.15	0.50	0.065	30
	1	235	550	8.9	24.7	3.12	36
	1	455	710	0.40	0.51	0.067	78
	1	610	790	0.69	0.63	0.082	107
Carbon tetrachloride	1	108	395	0.36	30.0	2.94	1
	1	164	475	8.2	35.2	3.45	23
	1	225	540	0.20	0.64	0.063	32
	2	610	790	0.55	0.565	0.055	97
Ethylene chloride ($C_2H_4Cl_2$)	1	168	475	3.15	85.5	16.8	4
	1	260	570	0.08	0.31	0.061	26
	1	260	570	3.60	7.55	1.48	47
	1	600	785	1.19	1.12	0.22	106
	1	567	770	1.62	1.58	0.31	103
Chlorobenzene (C_6H_5Cl)	1	257	570	0.17	0.36	0.14	48
	1	630	800	1.98	1.89	0.74	105

will condense before reaching the absorber if the gas is cooled to room temperature. The warm air from the heater causes an amount of evaporation and also a resultant temperature in the absorber somewhat higher than that caused by the colder air in the parallel line. This discrepancy can be compensated for by using a larger volume of solution in the "total sulfur" absorber.

A line-drawing recorder and a suitable switching mechanism give the conductivities of the two solutions at 1-minute intervals during the absorption period of 30 minutes. The increment of conductivity of the absorber receiving unheated gas represents sulfur dioxide; any additional increment in the other absorber represents other sulfur compounds, since the same calibration applies to both absorbers (see Figure 3). This calibration is based on different dilutions of standard sulfuric acid.

In Table I are summarized the results of analyses of known mixtures with air of the substances listed above.

Hydrogen sulfide of tested purity was displaced from a mercury pipet into a large measured air stream. The various liquids were handled in weighed Nesbitt bulbs, and they were volatilized by means of controlled air streams. The table also shows the wattage of the heater and the approximate exit temperature of gases. In all cases the air volume was 15 liters per minute. A current consumption of 250 watts was adequate to cause the oxidation of all these sulfur compounds to sulfur dioxide. The platinum wire was red and the gas temperature about 580° C. Even at 100 watts (390° C.) there was considerable oxidation of hydrogen sulfide, but the process was not quantitative. The reaction was nearly complete at 160 watts (470° C.).

Thiophene showed a distinct tendency to form sulfuric acid, and it was necessary to maintain the adapter at a higher temperature with this substance than with the others.

For the decomposition of the chlorine compounds it was necessary to use 500 to 600 watts. At 250 watts the yield of hydrochloric acid was only about 25 to 50 per cent. Evidently water vapor supplies the hydrogen to enable a substance like carbon tetrachloride to form hydrochloric acid (*3*). Some acid condensation in the adapter has been noted with the chlorine compounds, presumably as chloric or perchloric acids. At normalities below 10^{-3} all these acids, as well as sulfuric acid and nitric acid, have nearly the same conductivities, and practically the same calibration applies to them all. When large amounts of chlorine compounds were put through the heater at 400° to 600° C. some retention occurred in the silica tube, which caused a long continued low level evolution of hydrochloric acid when the temperature was raised to about 800° C. This condition is being investigated further.

The agreement between the theoretical and analytical concentrations is satisfactory. In many of these analyses sulfur dioxide was also present in variable amounts, but there is nothing to suggest that it interfered with any of the determinations. It was merely subtracted from the total sulfur or chlorine values. Some of the values in Table I represent the upper limit of the recording system under the chosen conditions of operation. Higher concentrations could readily be determined by changing the time or rate of sampling, or the cell constant.

The apparent lack of specificity of the proposed method suggests itself as a drawback. This difficulty can be resolved in some cases by preliminary absorption of certain constituents of mixtures. For example, hydrogen sulfide was absorbed by Reece, White, and Drinker (*4*) to enable

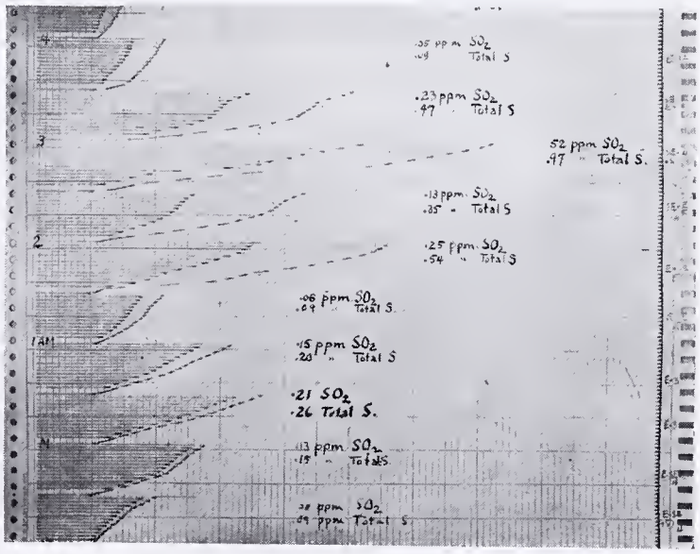

Figure 3. Portion of a Field Chart Showing Presence of Sulfur Dioxide with Other Sulfur Compounds

them to determine carbon disulfide. The hydrogen sulfide was then found by difference. Doubtless there is a practical limit to which the resolution of a complex mixture could be carried, but it would be entirely feasible to add another parallel combustion and absorption train to the assembly proposed here, by merely adding two more planes of ports to the valve.

The possibility of oxidizing nitrogen compounds to nitric acid, which would also be readily absorbed and determined, was explored for the most part without success. Ammonia was practically completely destroyed at 250 watts, but a trace of nitric acid was produced at higher temperatures. This destruction of ammonia would remove interference by this gas in the sulfur dioxide analysis (*2*). Pyridine, which interferes like ammonia, was not so completely destroyed in the heater. Hydrocyanic acid and aniline were without appreciable effect on either absorber, as was also nitrobenzene.

A unit of the sulfur dioxide–total sulfur apparatus has now been in continuous operation for several months in the field near an industrial district and has given a complete and satisfactory record. Figure 3 is a portion of the chart, showing the presence of sulfur dioxide, together with an approximately equal amount of other sulfur compounds, presumably hydrogen sulfide and possibly some mercaptans. It is improbable that any chlorine compounds were present.

Finally, it may be of interest to record a few observations on the physiological detection of some of these compounds. Under very favorable conditions, sulfur dioxide has been detected by sensitive persons at this laboratory at 0.3 p. p. m. by the sense of taste, and at 0.5 to 0.6 p. p. m. by smell. Hydrogen sulfide has a distinct odor at 0.3 p. p. m., and is detectable at 0.025 p. p. m. Ethyl mercaptan has a "very strong" odor at 0.6 p. p. m., and a distinct odor at 0.03 to 0.07 p. p. m. Its odor is detectable at approximately 0.002 p. p. m.

Summary

The automatic apparatus for the determination of small concentrations of sulfur dioxide in air has been modified by the substitution of a simple multiple-port stopcock-type valve for the numerous poppet valves of the older assembly. This equipment has also been modified to enable the simultaneous determination of the total volatile sulfur compounds in the air, with the same precision and sensitivity as characterized the sulfur dioxide analysis. Carbon disulfide, hydrogen sulfide, ethyl mercaptan, thiophenol, and thiophene have been so determined. The method is also applicable (at a higher operating temperature) to organic chlorine compounds, such as chloroform, carbon tetrachloride, ethylene chloride, and chlorobenzene. Nitrogen compounds could not be oxidized to nitric acid in this equipment.

Some observations are given on the physiological limits of detection of sulfur dioxide, hydrogen sulfide, and ethyl mercaptan.

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An Efficient Low-Holdup Laboratory Column

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IN THE hydrogenation studies on lignin being carried out in these laboratories a highly efficient, low-holdup laboratory fractionating column was urgently needed. Although the problem of devising such a column has been a common one throughout the history of organic chemistry, it has only been in the past few years that successful investigations have been carried out in this field. Of the several recent comprehensive articles concerning laboratory fractionation columns, those of Whitmore *et al.* (*11*) and Podbielniak (*8*) are the most important, since these two articles contain a critical discussion of all recently developed fractionating columns.

The specific problem faced in these laboratories was the separation of a mixture of aliphatic and cyclic alcohols (6 to 10 cc. in volume) which boiled over a range of 90° C. at 20 mm. to 180° C. at 1 mm. This required maximum theoretical plate efficiency accompanied by a total liquid holdup of less than 1.0 cc. Of the columns reported in the literature, the Purdue spiral screen (*6*) and the Podbielniak Heli-Grid (*8*) types of packing appeared to be the most satisfactory. The only objections to the use of these two designs were the difficult task of making a spiral screen with an outside diameter of 5 mm. or less, and the prohibitive cost of the excellent Podbielniak column.

Other excellent columns were considered inapplicable. The high-efficiency low-holdup column described by Selker, Burk, and Lankelma (concentric-tube packing, *9*) presents construction difficulties, requires a long time to attain equilibrium conditions (9 to 12 hours), and requires expensive temperature control equipment. The low-holdup spinning-band column developed by Baker, Barkenbus, and Roswell (*1*) has too low an efficiency (H. E. T. P., 7.8 cm.), while the efficient, small-diameter Stedman column (*2*) is difficult to construct and has a relatively high liquid holdup (0.18 cc. per plate).

A suggested alternative was the use of a type of packing proposed by D. F. Stedman of the National Research Council of Canada. According to this authority, an efficient packing for small-scale work should be obtainable by use of a narrow strip of wire gauze twisted to produce a series of vertical plates at an angle of 90° to each other. An investigation was carried out, therefore, on the applicability of such wire-gauze spirals as packings in fractionating columns.

Normal Fractionation Column

Wire Packing. After several attempts to prepare a satisfactory packing, the following method was adopted:

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A strip of 50 (or 60 × 40) mesh Monel metal wire gauze (1.5 times the length of the glass column) was cut from a sheet of this material with heavy scissors. The width of the gauze should be approximately two wire strands greater than the diameter (4 to 6 mm.) of the tube to be packed. The two outside longitudinal strands were then removed and the projecting lateral strands bent back in one direction until the strip fitted tightly in the glass column. The gauze strip was then ready for twisting. This was accomplished by use of two pairs of round-nosed pliers, the nose of one pair of which had been ground to a thickness of about 0.15 cm. (0.06 inch). The top of the gauze strip was gripped tightly with the thin-nosed pliers and 0.3 cm. (0.125 inch) away with the standard pliers, and the strip was then twisted through 90° with a simultaneous compression force, so that when the two pliers were at an angle of 90° they were touching each other. This resulted in the formation of two flat vertical planes at 90° to each other separated by two horizontal, opposed quadrants. The further twisting of the gauze strip was accomplished by gripping it just below the first bend with the thin-nosed pliers and 0.3 cm. (0.125 inch) below that point with the standard pliers. The twisting (in the same direction as the first turn) and compression motions were then carried out as described above. This entire process was repeated until the whole strip had been converted into a series of about 0.3-cm. (0.125-inch) vertical gauze platelets as shown in Figure 1.

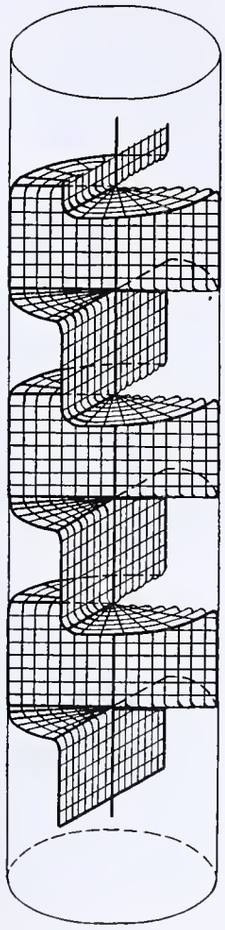

Figure 1. Column Packing

It is essential that the vertical axis of each platelet coincide with the line of axes of the adjoining platelets if a perfect fit is to be obtained, and it is therefore necessary to make certain that each platelet is properly centered before proceeding to the next. When the entire strip had been so treated it was found that the previously flattened horizontal strands had spread somewhat and that the strip no longer fitted the column. This was remedied by rolling the twisted strip back and forth on a hard flat surface and tapping gently with the flat side of a hammer until the packing just fitted the glass column. The simplest method for inserting the modified spiral was to lubricate it with oleic acid and pull it through the glass tube with the aid of a strand of copper wire tied to the end of the packing. The oleic acid was removed with ethanol and the copper wire dissolved in concentrated nitric acid.

If the packing has been properly constructed, a continuous film of liquid will be formed at the two lines of contact between the glass and the gauze when alcohol is poured down the column. If there are no more than a few (up to about five) breaks in the gauze-glass liquid film, the packing is considered satisfactory. If many such breaks occur, it is advisable to prepare a new packing. If the packing is made up of more than one continuous strip, it is not necessary to join the component strips, as two or more strips touching each other firmly have been found to give satisfactory results.

Glass Column. The glass portion of the fractionating column was of very simple design, as shown in Figure 2.

Although the sketch of the apparatus as used in these laboratories is drawn to scale, the only dimensions which are of significance are: (*a*) packed section, 5.0 ± 0.5 mm. in inside diameter; (*b*) cold-finger condenser, 10.0 ± 1.0 mm. in outside diameter; (*c*) reflux head, 15.0 ± 1.0 mm. in inside diameter. The delivery tube should be as small as possible, the limiting factor generally being that of the viscosity of the condensate. In this investigation tubing 5 mm. in inside diameter was found to be satisfactory. Although the packed sections of the columns constructed in these laboratories have varied in size from 37.5 to 112.5 cm. (15 to 45 inches) lengths of 60 to 90 cm. (24 to 36 inches) are most satisfactory with regard to ease of construction of the packing and total plate efficiency of the column.

The specially tapered and drilled ground-glass joint was made from a 12/30 male joint and was so constructed as to eliminate flooding at the joint. The flasks (still pots) were pear-shaped and it was found that 15-, 30-, 60-, and 100-cc. sizes were most convenient. All the flasks were equipped with 12/18 female grounds, so that the tapered end of the male ground projected below the closed area of the joint.

The still head was of the "partial condensation" type with cold-finger condenser. The inlet tube to the condenser was connected to a hot (90° C.) and cold water supply by means of a T-tube, so that it was possible to control the temperature of the condenser within the range of 20° to 90° C.

Adiabatic conditions were approximated by the use of an electrically heated vacuum jacket.

Column Heating Unit. The column was heated by means of a spiral of 8 mm. ($^1/_{32}$ inch) Nichrome ribbon wrapped uniformly around the vacuum-jacketed column, so as to produce a resistance of 12 to 20 ohms per foot. The Nichrome ribbon was carried beyond the packed, vacuum-jacketed portion of the column as far as the take-off side arm and the spirals were placed closer together in this area. The temperature outside the vacuum jacket was determined by means of a thermometer placed inside the air jacket.

The control circuit for the column heating unit consisted of a sensitive variable rheostat providing a range of 0 to 40 volts across the column heater. The bath consisted of an asbestos-wrapped, 400-cc. beaker wound with 24-gage (B. & S.) Nichrome wire (40 to 50 ohms) through which a variable resistance circuit operated, providing a range of 0 to 110 volts.

The fraction receiver, also shown in Figure 2, was a modifica-

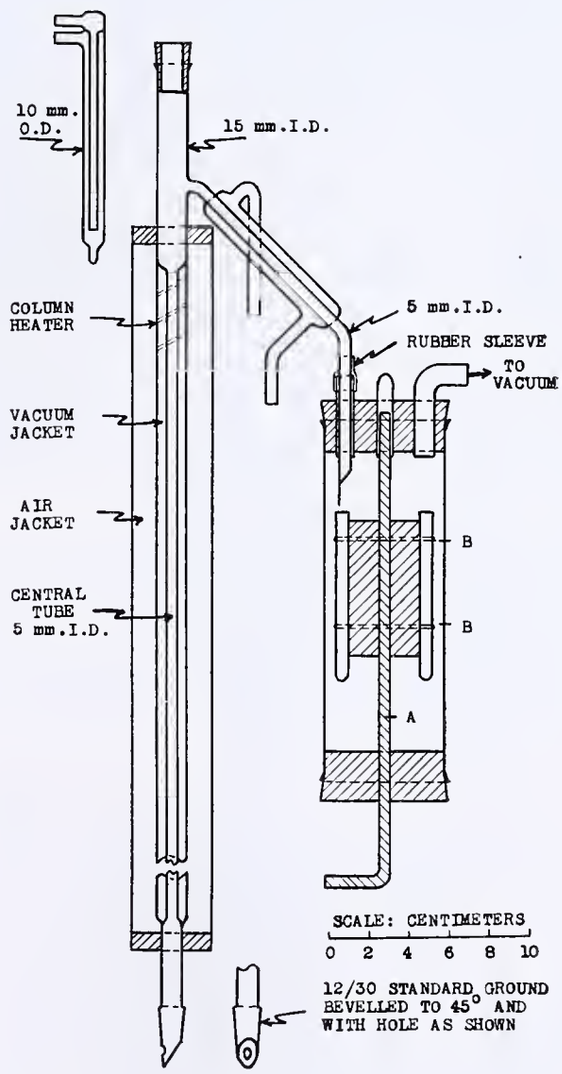

Figure 2. Fractionating Column, Condenser, and Receiver

tion of the Ellis type (*3*) in which the receiving tubes are held on the movable axis, *A*, at points *B-B* with rubber bands and the size of the tubes is governed by the size of the fractions to be collected (0.4 cm. in diameter × 5 cm. in length for 50- to 300-mg. samples, or 0.6 cm. × 8 cm. for 0.50- to 1.5-gram samples).

Operation of the Column. In an actual fractionation the procedure was divided into three stages—namely, flooding, establishing equilibrium conditions, and collecting fractions. In order to simplify the regulation of the bath and column temperatures during the equilibrium stage it was found advisable to make certain observations during the flooding operation. The following procedure proved most satisfactory:

The product to be fractionated (8 to 30 cc.) was placed in one of the still pots containing glass wool, this serving as an excellent antibumping agent at both atmospheric and reduced pressures. The contents of the flask were heated slowly by means of the electrically heated oil bath until distillate appeared in the lower 5 to 7.5 cm. (2 to 3 inches) of the packed section, whereupon the current passing through the bath heater was so adjusted that the bath temperature, T_1, remained constant. The voltage (or amperage, depending upon whether current control is maintained with a voltmeter in parallel or an ammeter in series with the bath heater) was recorded as V_1, since the bath temperature at this stage was generally the same as that used in the equilibrium stage. As soon as this voltage value was attained the current through the column heater was gradually increased from zero until the point at which the liquid film at the base of the packed section almost disappeared. The voltage across the column heater at this point was recorded as V_2.

Flooding was now accomplished by increasing the bath temperature rapidly (110 volts across the bath), the column voltage being maintained at V_2. When all the packing had been flooded the current across the bath heater was lowered to V_1. The bath was cooled to T_1 by inserting a Pyrex test tube containing cold water into the bath, and the bath surrounding the still pot was lowered cautiously in order to allow the packed section to drain slowly. There was so little tendency for the packing to dry during the draining operation that the bath frequently could be removed completely until the final traces of excess liquid had drained into the flask.

When all the excess liquid had drained from the packed section, the bath was raised again and the bath temperature then regulated to give a reflux rate (condensate return from drip point of cold finger) of approximately 30 drops per minute (38 to 40 cc. per hour) for 30 minutes in order to reach equilibrium. Although this period may appear short, test runs at total reflux showed an increase of less than one theoretical plate (23-plate column) when the time was extended to 90 minutes.

The actual fractionation was carried out either continuously or in batches. In the continuous method, which required careful control of the cold-finger temperature, distillate was collected continuously at a rate of about 100 to 200 mg. per hour. In the batch method 100-mg. samples of distillate were collected every 45 minutes by rapidly increasing the temperature of the cold finger. As soon as the sample appeared in the side arm the cold finger was cooled down to total-reflux temperature again and the system permitted to attain equilibrium (30 minutes at total reflux).

The column was found to operate satisfactorily either at atmospheric or reduced pressure, and materials boiling as high as 200° C. at 1 mm. could be distilled successfully.

Efficiency of the Column. The theoretical plate values on the column were determined by use of methylcyclohexane–*n*-heptane mixtures according to the procedure described by Ward (*10*). The liquid holdup was determined by the method of Fenske (*4*). The results of efficiency measurements on a pre-flooded 43-cm. (5 mm. in inside diameter) packed section are shown in Table I.

The flood point was found to be slightly more than 110 cc. per hour reflux (measured at top of column). This value was fairly constant at atmospheric or reduced pressures except where the viscosity of the liquid was relatively high.

Other columns, packed and operated as described above and having efficiencies of 28 to 45 plates (length of packing, 50 to 90 cm., 20 to 36 inches), have been used extensively in these laboratories.

Sample Fractionation. Beta-hydroxypropiovanillone was hydrogenated in dioxane solution over CuCrO at 280° C. Three grams of the product were fractionated in a Monel metal–packed, 51-cm. (20.5-inch), 28-plate column constructed as described above. The results are shown in Figure 3. The identity of the products (3-cyclohexylpropanol-1 and 4-*n*-propylcyclohexanol) was proved by preparation of their phenyl urethanes. The fractionation conditions were as follows: distillation pressure, 20 mm.; bath temperature, 120° to 150° C.; column temperature, 70° to 100° C.; reflux rate (at top of column) 30 drops per minute; total pressure drop at the above reflux rate, 0.7 mm. (0.025 mm. per plate); flood point, 110 cc. per hour reflux; take-off rate, 0.2 cc. per hour. When the quantity of residual, unfractionated material became less than the holdup of the column, it was forced over into the receiver (last point on fractionation curve) by raising the column temperature.

Summary. An efficient, easily constructed, low-holdup fractionating column having an H. E. T. P. of 1.88 cm. and a liquid holdup of 0.045 cc. per plate is described. This apparatus compares favorably with the larger, Purdue spiral-screen column (*6*), but is less efficient than the more expensive Podbielniak Heli-Grid column (*8*). Columns of varying lengths (5 mm. in diameter) having theoretical plate values from 23 (42.5 cm., 17 inches) to 45 (90 cm., 36 inches) have been constructed and employed for the fractionation of small quantities (6 to 15 cc.) of liquids boiling from 100° C. at 760 mm. to 200° C. at 1 mm.

Semimicro Fractionating Column

When wood meal is treated with ethanolic hydrogen chloride, a portion of the lignin is converted into a mixture of

Table I. Efficiency Tests on 43-Cm. (17-Inch) Fractionating Column

Reflux Rate (at Top of Column)		Theoretical Plates	H. E. T. P.		Operating Holdup
Cc./hour	*Drops/min.*		*Cm.*	*Inches*	*Cc./plate*
38.5	32	23	1.88	0.74	0.045
63.0	52	19	2.28	0.89	...
84.0	70	17	2.54	1.00	...
110.0	92	15.5	2.79	1.10	0.066

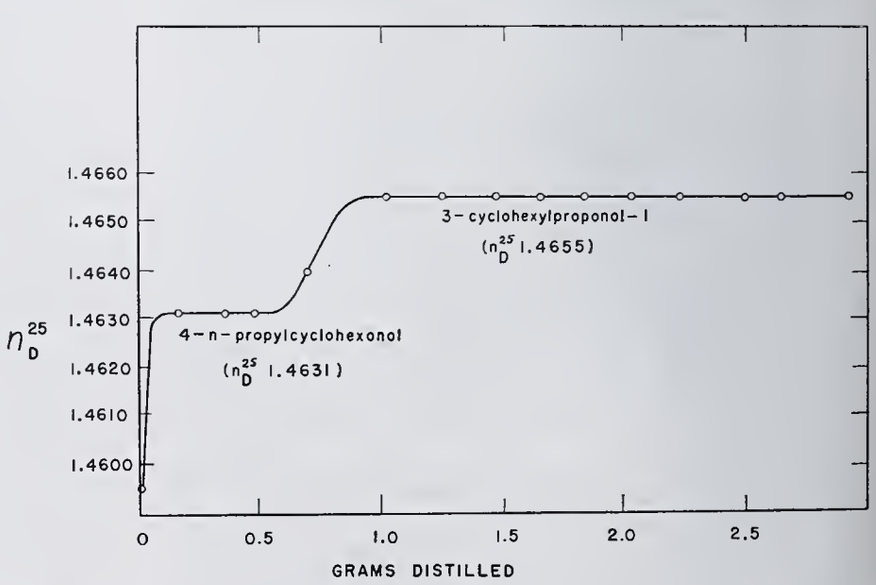

Figure 3. Fractionation Curve of Products from Hydrogenation of β-Hydroxypropiovanillone

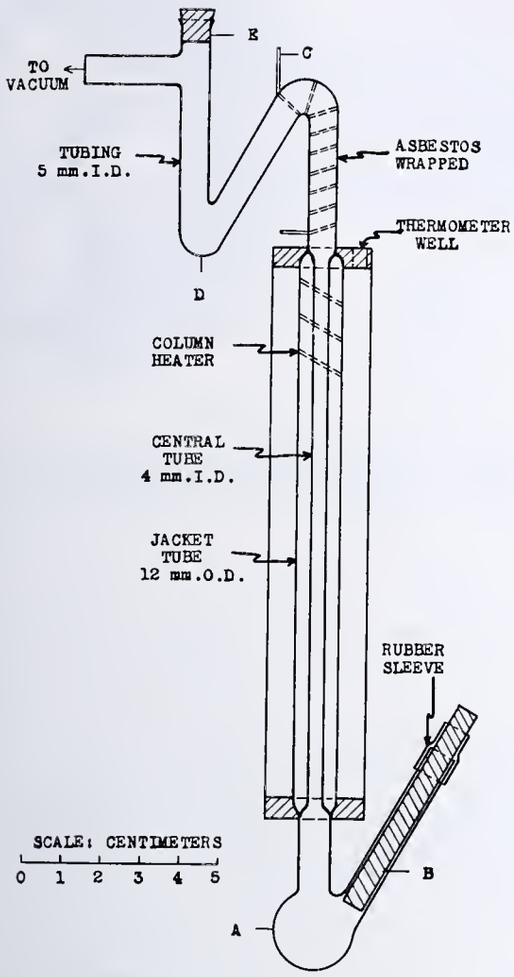

FIGURE 4. SEMIMICROCOLUMN

relatively high-boiling phenols, ketones, and aldehydes (*5*), which, in the past, have been laboriously separated on the basis of their chemical properties. Although the applicability of fractional distillation to this problem was considered frequently, there was no highly efficient apparatus available for the fractionation of very small quantities (0.5 to 1.5 cc.) of high-boiling (85° to 150° C. at 1 mm.) materials. [Since this article was submitted for publication it has been called to the authors' attention that a very efficient semimicrocolumn is described in a recent paper (*7*).]

The development of the packing described above, however, led to the construction of an efficient "semimicro" fractionating column of simple design and operation which has proved to be of great value in the separation of the phenolic and ketonic products obtained in the ethanolysis of wood meal. Examples of such fractionations using this semimicrocolumn will appear in forthcoming papers by Hibbert *et al.*

OPERATION OF THE COLUMN. It is unnecessary to give a detailed description of the construction of the column which is shown in Figure 4. The packing is described in detail above, and the Pyrex portion can be built by the average amateur glass-blower from the sketch.

The operation of the semimicrocolumn, although relatively simple, does require a fair degree of care if optimum results are to be attained.

The liquid sample (0.6 to 1.5 cc.) dissolved in a low-boiling solvent was placed in the bulb, *A*, together with a wad of glass wool. The side arm was closed with a glass rod plug, *B*, as shown in Figure 4 and the solvent was removed at atmospheric pressure. Until equilibrium was attained the procedure was the same as that described above—flooding, draining, then slow, controlled reflux for 30 minutes. The vapor was condensed for reflux by means of an air jet directed on the top of the column. The sample-collecting procedure was similar to the "batch" method described above. In order to collect a fraction, a current of approximately 1 ampere was sent through the 4-ohm heating unit, *C*, in the column head. As soon as distillate appeared in the side arm the current through *C* was shut off, the bath lowered, and the bath temperature raised. While the temperature of the bath was being raised to the flood point the fraction was removed from the receiver, *D*, as follows:

Air (or better, nitrogen) was admitted to the system and the liquid in *D* removed through side arm *E* to a tared test tube with the aid of a clean medicine dropper. The residual liquid in *D* and that remaining in the medicine dropper were washed into a second tared test tube by use of a pure low-boiling solvent (ether or ethanol). (In washing *D*, it was important that some solvent be forced back into the column in order to remove all traces of the first fraction from the condensing area.) After the washing operation the system was closed and re-evacuated, the bath was raised, and the procedure of flooding, draining, and refluxing was repeated.

The fractions obtained by this procedure, using the apparatus shown in Figure 4, weighed approximately 50 mg. If larger samples were desired—e. g., 100 mg.—the procedure was modified as follows:

After the first fraction had been forced over, the current in *C* was shut off, but the liquid in the pot was allowed to reflux at the same bath and column temperatures as were used prior to the collection of the first fraction. After refluxing had been continued for about 30 minutes, a second fraction was collected and the total contents of receiver *D* were removed as described above.

The column, as shown in Figure 4, was designed for the fractionation of high-boiling materials (85° to 150° C. at 1 mm.). For lower-boiling compounds (100° C. at 760 mm. to 100° C. at 10 mm.) it would be necessary to modify the head by removing the asbestos shield and to employ cooling (for total reflux) with a current of air. Both systems have been employed successfully in these laboratories. No plate determinations have been made on the semimicrocolumn, but on the basis of the previously described determinations on this new type of packing, the column shown in Figure 4 (13-cm. packed section) should have a 7-plate efficiency.

These "semimicro" columns are excellently suited for the final purification of "fractions" obtained from the larger columns described above. Frequently the "flat" portion of a fractionation curve (obtained from 10 to 15 cc. of a mixture) contained traces of lower- and higher-boiling impurities but the total volume of the fraction was too small (1 to 3 cc.) to permit refractionation. Such a material could often be purified readily by refractionation in the semimicrocolumn with a recovery as high as 80 to 90 per cent.

Acknowledgment

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A Colorimetric Method for the Determination of Sodium

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FOLLOWING the publication of the method for the gravimetric determination of sodium as a hydrated triple salt, $NaZn(UO_2)_3(C_2H_3O_2)_9 \cdot 6H_2O$, by Barber and Kolthoff (*1*) and the corresponding magnesium salt by Caley and Foulk (*3*), a number of colorimetric methods based on the color conferred on the solution by the uranyl ion have been described in the literature.

Caley and Foulk suggested dissolving the triple acetate in water and comparing the yellowish solutions colorimetrically. They mention that "best results are obtained when the solutions (known and unknown) show a fairly deep straw color". This is equivalent to saying that the sensitivity is limited.

It has been suggested by Barrenscheen and Messinger (*2*) that a water solution of the triple acetate treated with potassium ferrocyanide gives a stable brownish red color due to a potassium uranyl ferrocyanide which may be used as the basis of a colorimetric method. This procedure has been criticized by several authors on the basis that the color intensity varies with such external conditions as temperature and excesses of reagents.

Rosenheim and Daehr (*4*) while investigating the hydrates of uranium tetroxide found that if a solution of hydrogen peroxide is added to a solution containing the uranyl ion, a white amorphous precipitate forms which they took to be a hydrated uranium tetroxide. If, however, the solution is first made alkaline with sodium or ammonium carbonate and the hydrogen peroxide is then added, a true solution is formed which varies in color from an intense yellow to orange to red, depending upon the concentration of the uranyl ion.

It occurred to one of the authors that this color might be made the basis of a colorimetric method for determining sodium.

This method is basically only a method for estimating the triple acetate of sodium uranyl and zinc or magnesium acetate and therefore assumes all of the precautions and interferences of the methods of Barber and Kolthoff and of Caley and Foulk.

Preliminary experiments using a Duboscq colorimeter indicated that the intensity of color was somewhat dependent upon the amounts of ammonium carbonate and hydrogen peroxide used.

At this time a Coleman spectrophotometer became available and the transmission curves shown in Figure 1 were taken. Curve A shows the transmission of a solution prepared by treating an amount of the triple zinc acetate equivalent to 0.308 mg. of sodium, with an excess of 3 per cent hydrogen peroxide and saturated ammonium carbonate solutions, and diluting the whole to 25 ml. Curve B is the same curve for a solution containing an amount of triple acetate equivalent to 1.300 mg. of sodium, again diluting the whole to 25 ml. Both curves show transmissions of suitable size for wave lengths from 5200 Å. to about 5500 Å.

Therefore, using a 30 mμ fixed slit in the spectrophotometer, a band at 5200 Å. was chosen for all succeeding measurements.

The procedure involved making a standard triple acetate solution in a known weight of water and making comparison solutions from this by weight. The required amounts of 3 per cent hydrogen peroxide and saturated ammonium carbonate were added and the whole was made up to a volume of 25 ml. in calibrated flasks. It is important to add the ammonium carbonate solution first, followed by the hydrogen peroxide. If the order is reversed, the amorphous precipitate

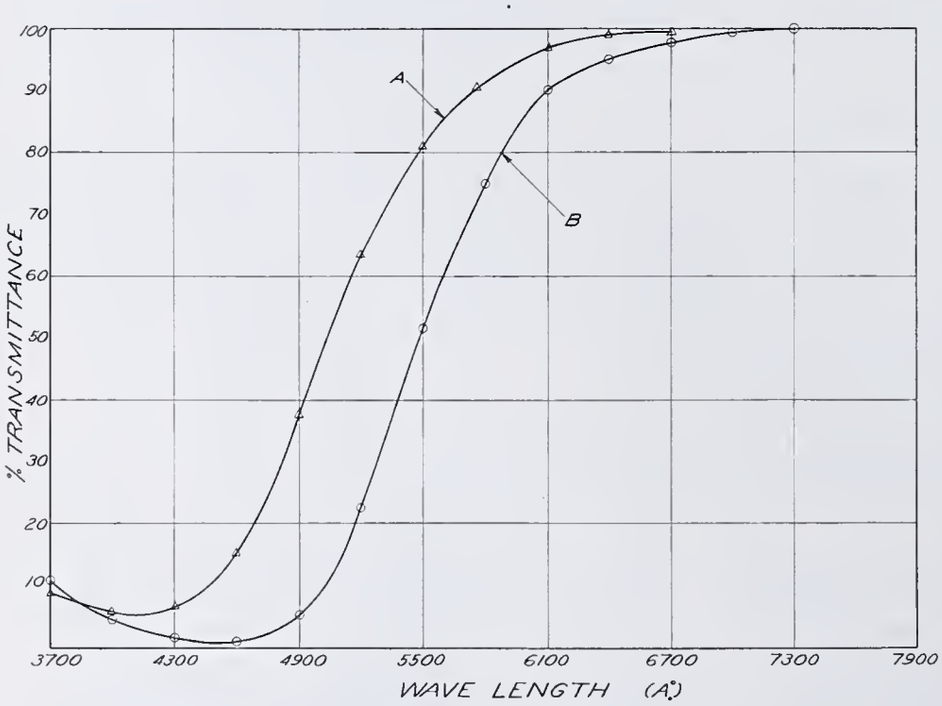

FIGURE 1

Table I. Effect of Ammonium Carbonate on Transmittance of Triple Acetate Solutions

Sodium Taken	Saturated $(NH_4)_2CO_3$	Transmittance
Mg.	*Ml.*	%
0.03	0.5	71.0
0.03	1.0	84.5
0.03	2.0	95.5
0.03	5.0	97.5
0.03	6.0	99.6
0.03	10.0	99.6
0.15	0.5	50.6
0.15	1.0	55.2
0.15	3.0	58.8
0.15	5.0	58.9
0.3	0.5	13.8
0.3	1.0	14.2
0.3	3.0	14.2
0.3	5.0	14.2

Table II. Effect of Hydrogen Peroxide on Transmittance of Triple Acetate Solutions

Sodium Taken	3% H_2O_2	Transmittance
Mg.	*Ml.*	%
0.15	0.5	12.7
0.15	1.0	13.7
0.15	3.0	14.8
0.15	5.0	14.9
0.03	0.5	58.6
0.03	1.0	63.8
0.03	5.0	63.8

referred to by Rosenheim and Daehr tends to form. Because the ordinary hydrogen peroxide of commerce stabilized with acetanilide was not satisfactory, a fresh 3 per cent solution of this reagent was made each time by diluting 30 per cent perhydrol.

Table I shows the effect of ammonium carbonate on the color. It can be seen that the transmittance varies somewhat with the amount of ammonium carbonate, but for the amounts of sodium taken for ordinary determinations, 6 ml. of a saturated solution seemed to be a sufficient excess to develop maximum color rapidly.

Table II shows the corresponding variation for the number of milliliters of 3 per cent hydrogen peroxide. The standard conditions were then chosen to give sufficient excess of each reagent—namely, 6 ml.

The following experiments were run to test the fading of the color:

The proper amounts of the stock solution of the triple acetate were weighed into 25-ml. volumetric flasks, to each flask separately the ammonium carbonate and hydrogen peroxide were added, the solution was diluted to volume and the color comparison was made immediately. About 5 minutes were required for this and for the necessary adjustments on the spectrophotometer, so that the readings taken at zero time were in error about 5 minutes.

Table III. Effect of Time on Transmittance of Triple Acetate Solutions

Sodium	Time after Development	Transmittance
Mg.	*Hour*	%
0.22	0	75.2
	2	75.1
0.55	0	51.2
	2	51.1
0.88	0	35.8
	2	35.9
1.02	0	30.9
	2	30.8
1.40	0	21.2
	1	21.3
	21	21.5
1.66	0	17.2
	1	17.3
	21	17.4
1.90	0	15.2
	1	15.0
	21	15.1
2.09	0	12.3
	1	12.1
	21	11.8

Table III shows the effect of time on the per cent transmittance. Within the time necessary to make a colorimetric determination, there is no appreciable fading. This appears to be true in spite of the fact that on standing for some time, the decomposition of the hydrogen peroxide is evidenced by the appearance of bubbles in the solution. But after shaking to remove the bubbles, the transmittance appears to be unchanged when measured in the spectrophotometer.

An attempt was made to determine the minimum amount of sodium distinguishable by this method, by making a number of pairs of solutions from the stock solution which differed from each other by only small amounts of sodium. Table IV shows some of these results. As the transmittance decreases the sensitivity decreases but, by choosing a more appropriate wave band for the redder colors, this could be improved.

Table IV. Sensitivity of Colorimetric Method

	Sodium	Transmittance
	Mg.	%
	0.308	63.2
	0.316	67.8
Difference	0.008	4.6
	0.675	47.2
	0.686	48.3
Difference	0.011	1.1
	1.24	23.2
	1.25	23.8
Difference	0.01	0.6

Figure 2 shows the logarithm of the transmittance and Table V shows the data from which this curve was obtained.

The few samples on which this method was tried out gave excellent results, as shown by Table VI.

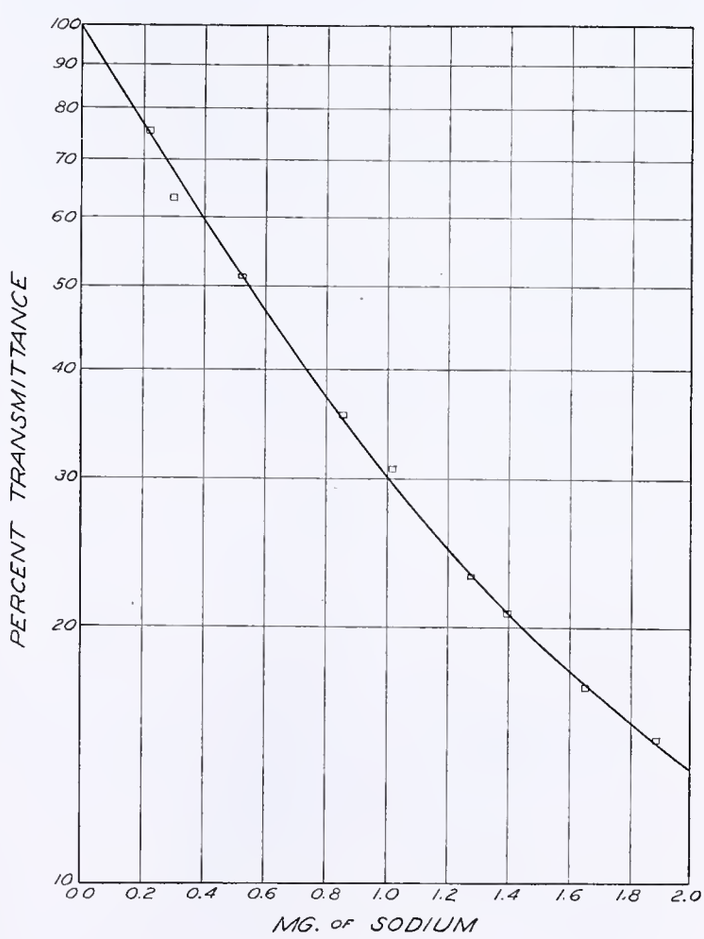

Figure 2

Table V. Transmittance

Sodium	Transmittance	Sodium	Transmittance
Mg.	%	*Mg.*	%
0.223	75.2	1.30	23.2
0.308	63.2	1.40	21.2
0.548	51.2	1.66	17.2
0.879	35.8	1.90	15.1
1.02	30.9	2.09	12.3

Table VI. Experimental Results

Sample	Na_2O Present	Na_2O Found
	%	%
B. S. flint clay 97	0.33	0.33
B. S. feldspar 70	2.38	2.36
B. S. opal glass 91	8.48	8.47

A few experiments showed that the triple magnesium acetate gave similar results, but no particular advantages were noted.

Summary

A colorimetric method for sodium has been developed which depends on treating a solution of the triple acetate, precipitated by the method of Barber and Kolthoff or of Caley and Foulk, with an excess of ammonium carbonate and hydrogen peroxide which develops an orange to red colored solution. When using a narrow wave band this color is proportional to the concentration of sodium ion in solution. The transmittance-concentration curve for these solutions has been run over the spectral range from 3700 Å. to 7700 Å. A wave band of 5200 Å. was chosen for this comparison. Various factors affecting the stability of the color were investigated and are reported.

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A Molecular Still Designed for Small Charges

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The active principle of marihuana is contained in a viscous, high-boiling oily mixture, the active components of which have not as yet been obtained in crystalline form (except cannin, *1*).

In working with material of this character, it is frequently desirable to distill small amounts of it under high vacuum, either for the purpose of fractionating or simply to separate it from nonvolatile matter.

The still described herein was devised to afford a convenient means for carrying out this operation. With the dimensions indicated, it has proved satisfactory for distilling from 0.25 to 5 grams of oil. Two fractions of distillate may be collected and the still residue need not exceed 0.15 gram. Transfer losses are avoided, since the distillate is collected directly in tubes in which it may be retained until used in subsequent operations. It does not pass through any ground joints, thus avoiding the possibility of contamination with lubricant.

No provision has been made for determination of the distillation temperature. In distillations of this type, ebullition does not take place. The temperature is not a constant, but a variable related to the pressure and the rate of distillation; hence it is more satisfactory to characterize products by such properties as refractive index, rotation, and the like.

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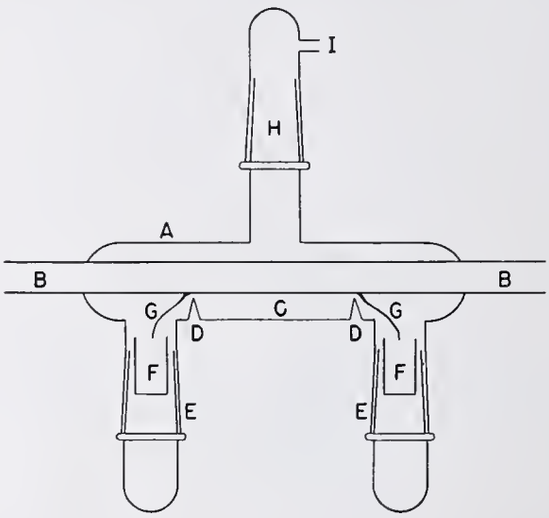

Apparatus

The apparatus is illustrated in the accompanying figure. It consists of a 35-mm. tube, *A*, 175 mm. in length, through which a 14-mm. tube, *B*, is sealed so that its axis lies about 6 mm. above that of the outer tube, *A*. The still pot, *C*, is formed by two transverse creases, *D*, in the outer tube, 45 mm. from each end, extending to within 3 mm. of the central tube. To the end chambers formed by the creases are sealed 24/40 ₮ ground joints, *E*, in which the receivers, *F*, are supported as indicated, by suitable lengths of glass tubing. To the central tube are sealed, as illustrated, lengths of glass rod, *G*, leading from a point outside the transverse creases, but as near them as possible, to a point from which distillate will drip directly into the receivers. Filling and cleaning are provided for by a tube, *H*, bearing a 24/40 ₮ joint sealed to the top of the still. From the closure of this tube a length of 8-mm. tubing, *I*, leads to the vacuum system.

Construction of the device involves considerable strains in the glass and the annealing accordingly must be thorough. The authors have found an ordinary muffle furnace convenient for the purpose.

Operation

In operation a suitable charge is placed in the still pot, and the apparatus is set level and gradually evacuated while the charge is gently warmed. After thorough degassing, the still is tipped (*2*) a little toward one end, full vacuum is applied, and the charge is heated until distillation proceeds at a suitable rate. Distillate accumulates on the condenser and follows it to the sealed-on glass rod, whence it is diverted to the receiver. The condenser is maintained at such a temperature that distillate flows along it smoothly and does not tend to form large drops which might fall off outside the still pot but before reaching the tip which carries it to the receiver. After one fraction has been collected, the still is tipped toward the other end and the second fraction collected in the same way.

The still has been successfully heated by means of a small flame placed some distance below it. A properly designed electric heater might prove more satisfactory.

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Determining Magnesium in Plants and Soils

Adaptation of the 8-Hydroxyquinolate Micromethod

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THE use of 8-hydroxyquinoline in the precipitation and determination of magnesium has received considerable attention, particularly since several micromethods for the estimation of hydroxyquinolate have been found satisfactory (*1, 4, 5, 9*). The general methods of estimation include oxidation with hexanitrato-cerate in perchloric acid solution and color reactions involving the use of ferric salts or the Folin phenol reagent. A comparison of certain of these methods of estimation has recently been reported by Gerber *et al.* (*1*). Magnesium hydroxyquinolate has a constant composition and is very sensitive if interfering ions are eliminated.

The determination of magnesium as the phosphate proved entirely unsatisfactory in a system of soil and plant analysis involving use of the more rapid and sensitive micromethods and for this reason several of the hydroxyquinolate procedures were studied (*8*). Preliminary studies using plant ash and synthetic solutions indicated that the hydroxyquinolate method is sensitive at low magnesium concentrations, even in the presence of considerable amounts of ammonium salts. However, great care is necessary for best results, and certain precautions must be rigidly observed. Consequently the procedure adopted is given in considerable detail and reasons for certain precautions are indicated.

Preparation of Solution

PLANT MATERIAL. Oxidize 2 grams of the air-dry material, ground to pass a No. 20 A. S. T. M. screen, in a 100-ml. beaker by the method of Gieseking *et al.* (*3*). For ordinary plant material it is not necessary to treat with nitric acid before adding the mixed nitric and perchloric acid solutions. Oily seeds, such as corn grain may be safely oxidized with perchloric acid if the following procedure is used.

Dry 20 grams of the material at 100° C. and extract several times with anhydrous ether to remove fatty substances that would react too violently with perchloric acid. Treat the extracted material in a 600-ml. beaker on a steam hot plate with 20 ml. of concentrated nitric acid. If considerable frothing occurs, stir vigorously a few times with a stirring rod. After a minute or two when reaction subsides somewhat, cover with a watch glass to prevent too rapid evaporation and allow the mixture to evaporate to a thick yellow paste. Add 30 to 40 ml. more of nitric acid and leave on the steam plate several hours or overnight. Take up the residue with 20 ml. of a mixture of 2 parts of nitric acid and 1 part of perchloric acid, transfer to a gas or electric hot plate, and heat carefully at 140° to 160° C. until the solution becomes clear. If the material becomes dark brown or black during this heating, add more nitric acid, a few milliliters at a time. Usually, however, the solution boils down gently and clears up without trouble.

After the solution clears, remove the watch glass and allow most of the excess perchloric acid to evaporate. Take up the residue in 10 ml. of 1 to 1 hydrochloric acid, heat on the steam hot plate for 20 minutes, and filter through a close-textured filter paper. Wash the filter six times with hot 10 per cent hydrochloric acid and three or four times with water. Transfer the filtrate to a 50-ml. volumetric flask, dilute to volume, and mix thoroughly. Take measured portions of this solution for analysis.

SOIL. Several common methods employed in soil analysis for obtaining solutions of total and replaceable bases have been used and will not be described in detail. After the solution is taken to dryness to destroy organic matter or dehydrate silica, the residue is taken up with 10 ml. of 1 to 1 hydrochloric acid in the same way as described for plant materials, filtered, and transferred to a 50-ml. volumetric flask.

Reagents and Solutions

QUINOLATE REAGENT. Dissolve 1 gram of 8-hydroxyquinoline in 50 ml. of 95 per cent alcohol. Prepare fresh every 2 or 3 days. One milliliter of this solution will precipitate approximately 1.5 mg. of magnesium.

FERRIC CHLORIDE–ACETIC ACID REAGENT. Dissolve exactly 10 grams of ferric chloride hexahydrate in 2 liters of water containing 10 ml. of glacial acetic acid.

WASH SOLUTION. Mix 1 part of 95 per cent alcohol and 1 part of 10 per cent ammonium hydroxide. Keep in a well-stoppered bottle.

STANDARD MAGNESIUM SULFATE SOLUTION. Dissolve in distilled water 12.038 grams of magnesium sulfate that have been recrystallized from A. C. S. grade magnesium sulfate heptahydrate and dehydrated at a temperature above 200° C., and dilute to 2 liters. This solution after mixing is 0.1 *N*. A 0.01 *N* solution is prepared by diluting 50 ml. of the standard solution to 500 ml.

Precipitation of Magnesium

Transfer a measured portion of the solution to be analyzed containing 0.1 to 0.7 mg. of magnesium to a 15-ml. conical centrifuge tube, add 5 drops of glacial acetic acid and 1 drop of methyl red indicator, and mix thoroughly. If the tube is not over half full, mixing can be effected most rapidly by holding the tube near the top firmly between the thumb and forefinger of one hand while the fingers of the other hand are flipped rapidly against the bottom of the tube. Add 1 to 1 ammonium hydroxide carefully from a buret with frequent mixing until the solution turns from red to a very faint red or pink (not yellow) corresponding to about pH 6.0. Add 2 or 3 drops of dilute 1 to 4 acetic acid to give a more distinct red. The solution should finally be between pH 5.0 and 5.3. Add 3 drops of hydroxyquinolate reagent, mix, and place the tube in a beaker of hot water for 10 to 15 minutes to precipitate iron and aluminum. Centrifuge while still hot at 2000 r. p. m. for 10 minutes and pour the supernatant liquid carefully into a second 15-ml. centrifuge tube. Wash the sides of the first tube carefully with a thin stream of warm water from a 2-ml. pipet, stir up the precipitate by the method described above, centrifuge, and transfer the washings to the second tube.

To precipitate calcium add 1 ml. of a saturated solution of ammonium oxalate to the second tube and mix thoroughly. Place the tube upright in a beaker of hot water and heat on a steam hot plate for 30 minutes. Remove the tube from the beaker and place it in a rack to stand 2 to 4 hours. Add 5 or 6 drops of 95 per cent alcohol to the tube to break up any film of calcium oxalate that might have formed at the liquid surface and centrifuge for 10 minutes at 2000 r. p. m. Transfer the supernatant liquid containing magnesium to a third 15-ml. conical centrifuge tube by carefully pouring it over. Wash the calcium oxalate precipitate twice with 2-ml. portions of the alcohol-ammonia wash solution, using the same procedure as for the iron and aluminum precipitate. Centrifuge and add the washings to the tube containing the magnesium solution. If desired, calcium may be determined in the usual way.

To the solution in the third tube, add 0.5 ml. of hydroxyquinolate reagent, or if more than 0.7 mg. of magnesium is present, use 1 ml. Stir thoroughly, this time with a stirring rod, since the tube is too nearly full to mix in the usual way. Add 2 ml. of concentrated ammonia, stir thoroughly, remove the stirring rod, and wash it with a fine stream of water. When precipitation starts, usually within a minute or two, cover the surface of the liquid with a layer of alcohol 1 cm. thick. Put the tube in a beaker of hot water and place on the steam hot plate for 20 minutes. Remove the tube from the hot water bath, stopper with a No. 1 rubber stopper, and place in a rack to stand 1 to 2 hours or overnight if convenient. Be sure the layer of alcohol does not evaporate entirely; this is important because the magnesium hydroxyquinolate precipitate has a strong tendency to creep up the sides of the glass and form a film at the surface of the liquid that will not centrifuge down.

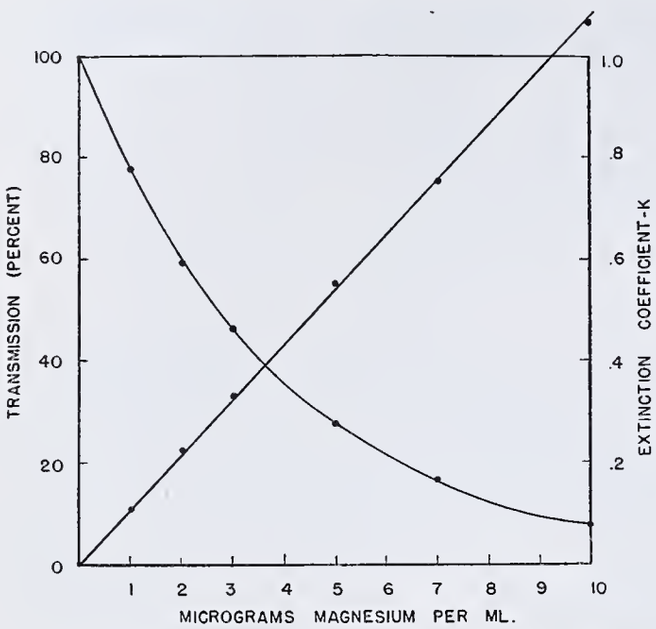

FIGURE 1. CONCENTRATION CURVE FOR MAGNESIUM
In acetic acid solution of magnesium hydroxyquinolate. Wave length 650 mμ, entrance slit 1.5 mm., exit slit 5 mμ, cell length 1 cm.

Centrifuge at 2000 r. p. m. for 10 minutes and draw off the liquid with gentle suction, using a tube with the tip drawn out to a capillary and turned up about 1 to 2 mm., so that the liquid can be drawn off without disturbing the precipitate. Wash the precipitate twice with 2-ml. portions of the alcohol-ammonia wash solution, covering the suspension each time with a layer of alcohol. Centrifuge and draw off the supernatant liquid as described above. Dry the precipitate carefully for a few minutes by placing the tube in a beaker of hot water on a steam hot plate, but do not leave so long that the precipitate becomes hard and dissolves too slowly. Remove from the steam bath, add 10 ml. of the ferric chloride–acetic acid reagent to the tube, and break up the precipitate with a small glass stirring rod to hasten solution, or stopper and shake vigorously. Solution is ordinarily complete and full color developed in about 0.5 hour.

Transfer an appropriate portion, usually 2 ml., of the colored solution, to a test tube, dilute with 10 ml. of the ferric chloride–acetic acid reagent, mix, and determine the light transmission by means of a photoelectric colorimeter or spectrophotometer (a Cenco-Sheard spectrophotelometer was used in this study). From the transmission value determine the magnesium content by a standard curve prepared by precipitating 1- to 10-ml. portions of a 0.01 N magnesium sulfate solution by the method described above.

A standard curve at a wave length of 650 millimicrons is shown in Figure 1. The percentage of magnesium in an unknown sample is calculated by picking the amount of magnesium in micrograms from the curve corresponding to the per cent transmission and applying the following formula:

$$\frac{\text{Micrograms} \times \text{dilution}}{\text{Weight of sample} \times 1{,}000{,}000} \times 100 = \text{per cent magnesium}$$

Figure 2 shows a light-absorption curve for the color developed by magnesium hydroxyquinolate in ferric chloride–acetic acid solution. In this curve values for specific extinction are plotted against wave length in millimicrons. The following application of the Beer-Lambert law was used (*2*, page 75):

$$\log_{10}\left(\frac{I_0}{I}\right) = kcl$$

$$k = \frac{\log_{10}\left(\frac{I_0}{I}\right)}{cl}$$

$$K = \frac{\log_{10}\left(\frac{I_0}{I}\right)}{l}$$

where
I_0 and I = intensity of light at a given wave length transmitted by solvent and solution, respectively
k = specific extinction
c = concentration of magnesium in mg. per ml.
l = length of cell in cm.
K = extinction coefficient

This curve shows maximum light absorption at a wave length of 660 millimicrons with a secondary maximum at 460 millimicrons. These maxima have been found to hold at widely different concentrations. Agreement with Beer's law is indicated in Figure 1, where extinction coefficients or K values are plotted against concentration. In this case a line passing through the origin falls on or very near to all points from 1 to 10 micrograms per milliliter.

The values for maximum absorption obtained in this study do not agree exactly with those reported by Gerber *et al.* (*1*), who found maxima at 650 and 425 millimicrons. The discrepancies in these values may be due to one or all of the following reasons: differences in the width of the wave band used, calibration of the instrument to proper wave lengths, or differences in the concentration of iron chloride. In the "spectrophotelometer" used in this study, a 1-mm. entrance slit and 2.5-millimicron exit slit was used. At this setting, owing to the side bands of light from the entrance slit, the total wave band isolated is 6.5 millimicrons in width (*6*). The instrument was calibrated from time to time with a fluorescent lamp at a wave length of 546 millimicrons. It was found in early work with this method that the concen-

TABLE I. EFFECT OF PHOSPHATE ION ON PRECIPITATION OF MAGNESIUM BY 8-HYDROXYQUINOLINE

0.01 N $MgSO_4$	Light Transmission, Wave Length 650 Millimicrons: No phosphorus	0.6 mg. of phosphorus	Difference
Ml.	%	%	%
1	78.5	77.0	−1.9
2	59.7	59.5	−0.3
3	46.5	46.8	+0.6
5	28.5	28.0	−1.8
7	18.2	18.0	−1.1

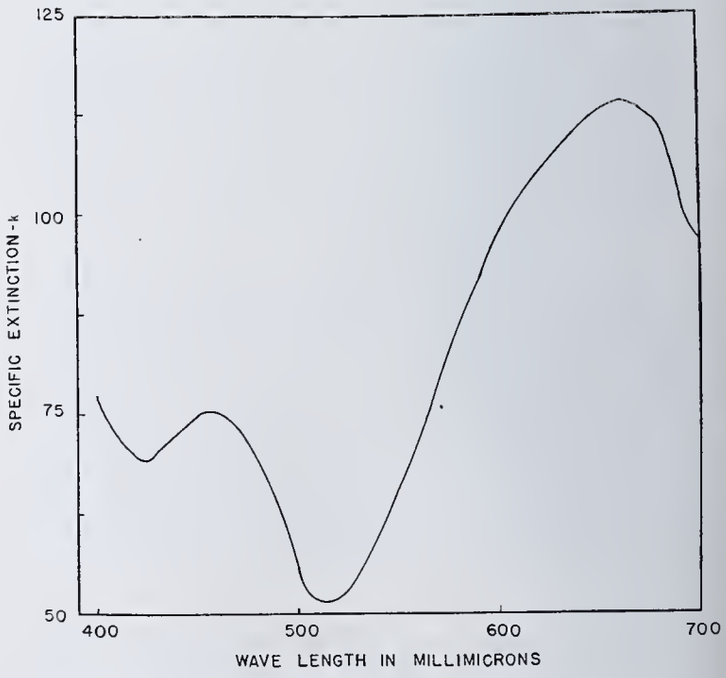

FIGURE 2. LIGHT ABSORPTION CURVE FOR MAGNESIUM QUINOLATE
1-cm. cell with 1-mm. entrance slit and 2.5 mμ exit slit

tration of iron chloride in the reagent has a great effect on the color produced. This effect is of sufficient importance to necessitate some care in preparing the ferric chloride–acetic acid reagent. Each lot of ferric chloride should be checked and kept tightly covered when not in use to prevent change in moisture content.

The concentration curves used in this work were drawn at 650 and 560 millimicrons. At these wave lengths it was unnecessary to use the red filter to prevent stray light in the instrument, and reference readings, I_0 values, of 100 per cent were more easily obtained.

Interfering Substances

The important cations interfering in the precipitation of magnesium hydroxyquinolate are iron, aluminum, and calcium. Iron and aluminum are usually separated by ammonium hydroxide or ammonium acetate at about pH 6.2. It was found, however, that these ions could be separated most easily and completely by precipitation with 8-hydroxyquinoline in acetic acid solution at about pH 5.3. Calcium is completely separated as the oxalate and thus eliminated. Since the procedure does not remove the phosphate ion, an experiment was set up to find out whether this ion interferes in the precipitation. Two sets of standard magnesium solutions were prepared: one with and one without the addition of 0.6 mg. of phosphorus, corresponding to the maximum amounts present in ordinary analysis. Magnesium was precipitated in both sets. The results shown in Table I indicate that the phosphate ion has little if any effect on the quantitative precipitation of magnesium quinolate. In all but one instance (values for 1 ml. of 0.01 N magnesium sulfate) differences were considered within the limit of error at the time the experiment was conducted. The effect of the 0.6 mg. of phosphorus on the precipitation of magnesium at low concentrations was rechecked at a wave length of 560 millimicrons, so values are not strictly comparable with those given in Table I, but at this wave length the values agreed within 0.5 per cent. It is true, however, that where 1.2 mg. of phosphorus were added, the precipitation of magnesium in amounts below 0.1 mg. was retarded. This is not considered particularly important since 0.1 mg. is at the lowest magnesium level for the accuracy of the method and the phosphorus level is higher than is normally encountered in ordinary analysis.

Table II. Recovery of Added Magnesium in Wheat Tops

(0.103 mg. of magnesium added)

Sample No.	Found in Sample	Theoretically Present	Actually Determined	Recovery of Added Magnesium	
	Mg.	*Mg.*	*Mg.*	*Mg.*	%
74	0.433	0.536	0.537	0.104	101
75	0.406	0.509	0.514	0.108	105
76	0.413	0.516	0.510	0.097	94
77	0.459	0.562	0.554	0.095	92
78	0.459	0.562	0.562	0.103	100
79	0.434	0.537	0.535	0.101	98
80	0.400	0.503	0.513	0.113	110
81	0.469	0.572	0.570	0.101	98
82	0.462	0.565	0.564	0.102	99
83	0.429	0.532	0.533	0.104	101

Accuracy of the Method

The method was checked for accuracy in three ways.

1. Known amounts of magnesium sulfate were added to one of duplicate portions of solution from ten ashed samples of wheat tops grown in a greenhouse experiment, and after determining magnesium in these aliquots, the percentage recovery was calculated. The recovery averaged 99.7 per cent for the ten samples. The data are presented in Table II.

2. Seventeen samples of corn grain were analyzed for magnesium by the above method and by a phosphate method similar to that described by Wall (7). A comparison of the results by the two methods indicates that the 8-hydroxyquinoline method gave an average magnesium content for all samples slightly higher than the phosphate method. The averages for the two methods were 0.113 and 0.111 per cent, respectively. The maximum difference between the methods for any one sample was 12 per cent and four of the seventeen samples gave differences greater than 3.8 per cent. These results are based on single determinations.

3. The base-exchange capacities of two soils were determined by saturating them with magnesium and measuring the amount of this ion taken up by the exchange complex after replacing with ammonium acetate and determining magnesium in the extracts by both the quinolate and phosphate methods. The base-exchange capacities of these soils had previously been determined by the calcium absorption method.

The results obtained by the three methods are shown in Table III. Good agreement was obtained in all cases, with the possible exception of the value 8.6 obtained by the magnesium phosphate method, and this value is within 5 per cent. All values are an average of duplicate determinations.

The method has been used by two analysts in determining the magnesium content of several hundred samples of plant material and soil extracts. In most of these a difference of 6 per cent between duplicate determinations was allowed. It was found necessary on the average to rerun one sample in twenty. The average difference between duplicates was within 2.5 per cent.

Table III. Base-Exchange Capacities of Two Soils Determined by Calcium and Magnesium Absorption Methods

Soil Type	Base-Exchange Capacity		
	Calcium determined as oxalate	Magnesium determined as quinolate	Magnesium determined as phosphate
	M. e. per 100 grams of soil		
Maury silt loam	17.6	17.7	17.5
Unclassified silt loam[a]	8.2	8.2	8.6

[a] From Berea Soil Experiment Field.

Summary

A micromethod for magnesium based on precipitation with 8-hydroxyquinoline was studied and adapted to the determination of this element in soils and plant materials. Precipitation and determination were found to be most satisfactory and reproducible when the amount of magnesium present in the test solution ranged between 0.1 and 0.7 mg. Magnesium was accurately estimated by the green color of iron hydroxyquinolate in acetic acid solution. The method is capable of a high degree of accuracy and reproducibility.

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Microextraction and Microtitration of Fatty Acids

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THE difficulties inherent in the titration of minute amounts of the higher fatty acids with alkali arise from two main sources: The end point, on the alkaline side of neutrality, is affected by absorbed carbon dioxide; this is particularly manifest when dilute reagents are used. The tint of the indicator at the end point is dependent upon the composition of the solvent, which is usually an alcohol-water mixture.

TABLE I. DUPLICATE STANDARDIZATIONS OF ALCOHOLIC ALKALI AGAINST STEARIC ACID ON THREE SUCCESSIVE DAYS

	Stearic Acid	Displacement	Normality
	Mg.	*Mm.*	
A	16.543	10.80	0.1699
	10.056	6.56	0.1700
B	13.679	9.34	0.1625
	13.999	9.54	0.1628
C	13.131	9.06	0.1608
	13.092	8.99	0.1615

In an attempt to minimize these difficulties, samples of fatty acids weighing 8 to 20 mg. have been satisfactorily titrated, using relatively small volumes of fairly concentrated alkali (0.16 *N*). Both the acid and alkali were dissolved in 90 per cent methanol, to obviate any variation in the composition of the solvent. α-Naphtholphthalein (*3*), 0.5 per cent solution in methanol, has proved a satisfactory indicator, and a micrometer-driven microburet of the type described by Scholander (*2*) has been used to deliver the alkali. The titration mixture was stirred by means of a long stainless steel wire fastened to the blade of an electric buzzer.

Alkali, approximately 0.16 *N*, was prepared by dissolving appropriate amounts of solid sodium hydroxide in freshly boiled 90 per cent methanol. This solution was well mixed and then allowed to stand, to permit settling of the sediment. Solvent for the acid to be titrated was made up by adding 10 drops of indicator solution to 10 ml. of 90 per cent methanol, boiling for a minute or so, and then adding sufficient alkali from the microburet to produce an olive green color. Two to 4 ml. of this solvent mixture were used to dissolve each sample of fatty acid, and the titration was carried out as rapidly as practicable. The end point taken was the match of the original solvent mixture.

Fading of the indicator on standing, due to absorption of carbon dioxide, was noted. After a half hour the change in the color of the solvent was so marked as to warrant its being discarded. However, it was felt that only small errors would be introduced if each titration was carried out to the same tint as the solvent mixture at that time. There was obviously no necessity for a blank titration.

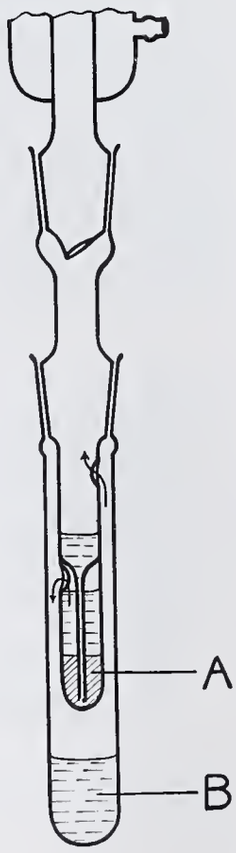

FIGURE 1. LIQUID MICROEXTRACTOR

The authors' buret, calibrated according to Scholander, delivered 3.171×10^{-2} ml. per mm. displacement.

For purposes of standardization, samples of pure stearic acid were weighed on the microbalance into ordinary 15-ml. centrifuge tubes. Duplicate standardizations of alkali on successive days showed a decrease in normality, indicating the necessity of daily standardization (Table I).

In view of the fact that this technique was investigated for the purpose of determining the total (free plus esterified) fatty acids in plasma, a liquid microextractor was employed (Figure 1). Into the lower chamber, *A*, which had a capacity of about 5 ml., 13.314 mg. of stearic acid were transferred in 1 ml. of ethanol and 1 ml. of 3 *N* alcoholic hydrochloric acid was added. In tube *B* were placed 10 ml. of petroleum ether, previously distilled from alkali, a reflux condenser was attached, and the whole assembly was set on the steam bath to extract for one hour. Tube *B* was then disconnected, and after evaporation of the petroleum ether, was kept overnight in vacuo over solid alkali, to remove traces of volatile acids. The residue was dissolved in neutralized alcohol and titrated precisely as described above. Standardization of alkali: 15.490 mg. of stearic acid required 10.66-mm. displacement, $N = 0.1613$. Titration of extract: acid required 9.16-mm. displacement

$$9.16 \times 3.171 \times 10^{-2} \times 0.1613 \times 284.3 =$$
13.32 mg. stearic acid recovered, corresponding to 100.1 per cent

In the analysis of plasma samples, lipides were extracted from 5-ml. samples of plasma by the alcohol-ether method of Bloor (*1*). The clear alcohol-ether filtrate was taken to dryness under a bell jar in a stream of nitrogen and the residue redissolved in ether. The solution was filtered into a small centrifuge tube and evaporated to dryness. The residue, in 1 to 2 ml. of ethanol, was transferred to chamber *A*, of the extractor with the aid of a long slender pipet; 0.2 ml. of 2 *N* sodium hydroxide in methanol was added and the mixture was gently heated on the steam bath until all the solvent had boiled away. Then 0.4 ml. of 2 *N* aqueous hydrochloric acid and 1 ml. of ethanol were added, tube *B* containing 10 ml. of petroleum ether was fitted on, and extraction under reflux was carried out on the steam bath. The extract, in tube *B*, was dried and titrated as described. Duplicate analyses on three different samples of plasma are given in Table II.

TABLE II. DUPLICATE ANALYSES OF PLASMA FATTY ACIDS IN THREE DIFFERENT SAMPLES

Sample	Fatty Acids	
	Microequivalents per ml.	
A	8.31	8.30
B	6.46	6.38
C	7.77	7.66

Summary

A method for the titration of 8- to 20-mg. samples of fatty acids has been described. The microestimation of plasma fatty acids is feasible by this method, in conjunction with a suitable extraction procedure.

Acknowledgment

This work was carried out with the aid of a grant from the Josiah Macy, Jr., Foundation.

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INDUSTRIAL AND ENGINEERING CHEMISTRY

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Physical-Chemical Method for Determination of Vitamins D in Fish Liver Oils

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SEVERAL methods for the physical-chemical determination of vitamins D have been studied and reported on in recent years. Some of these have given good results on nearly pure samples of vitamins D_2 and D_3. In the case of fish liver oils (D_3), agreement with the bioassay values has been obtained only over limited concentration ranges for a small number of oils. Further study therefore seemed warranted.

Reerink and van Wijk (*16*) and later Töpelmann and Schuhknecht (*22*) were successful in determining the concentration of vitamin D_2 (irradiated ergosterol) by measuring the height of the ultraviolet absorption maximum at 265 mμ. Ol Khin (*14*) also used this method in determining vitamin D_2 in irradiation products. On the basis of the work of Brockmann (*1, 2*) that the molecular extinctions of the two vitamin forms were the same, Marcussen (*11*) applied the measurement at this maximum to the determination of both vitamins D_2 and D_3. In the case of fish liver oil D_3, Marcussen first saponified the oil, then removed vitamin A, carotenoids, inactivated sterols, and other substances showing absorption in the region of 265 mμ by passing a heptane solution of the nonsaponifiable fraction through a Tswett column filled with Hydraffin K_4 as adsorbent. Thereby, he separated the vitamin D_3 in the percolate. The concentration of vitamins D in this solution was then obtained spectrographically. Calciferol (British Drug House) was used as the standard for converting the E (1 per cent, 1 cm.) (the extinction, $\log_{10} I_0/I$, for a 1 per cent solution in a cell of 1-cm. thickness) of the fish liver oil to units per gram of oil. Marcussen gave values for two tuna liver oils, one halibut liver oil, and one oil solution of an irradiated product.

Halden and Tzoni (*8, 9, 24*) obtained quantitative results with pure calciferol (D_2) based on a color reaction formed by heating a solution of the vitamin and pyrogallol in benzene, petroleum ether, or chloroform with a fresh solution of anhydrous aluminum chloride in absolute alcohol. The deep violet color formed was dissolved in absolute alcohol, giving a lilac-red solution suitable for colorimetric measurements. In the determinations of fish liver oils, it was found necessary to remove vitamin A. Cholesterol, ergosterol, and lumisterol did not interfere, but some of the irradiation products of ergosterol did give this color reaction.

Shear (*20*) suggested a colorimetric method based on the red color given by vitamins D in liver oils with a mixture of aniline and hydrochloric acid. Levine (*10*) reported that this color reaction was not specific for vitamins D.

Stoeltzner (*21*) proposed a colorimetric method based on the formation of color when phosphorus pentachloride was added to an oil solution of vitamins D. However, this reaction was found by Christensen (*4*) to be typical of other compounds present in natural oils.

Rutkovskiĭ (*19*) showed that the green color of the Tortelli-Jaffee reaction (*23*), formed when an acetic acid solution of vitamins D was mixed with a 2 per cent solution of bromine in chloroform, was also characteristic of the provitamins.

Robinson (*18*) reported a colorimetric method based on the yellow color formed when an alcohol solution of the vitamins D was boiled with sodium nitrite and acetic acid, then made slightly alkaline. This reaction was not given by cholesterol, ergosterol, dehydroergosterol, or lumisterol, but vitamin A gave a strong orange color and the nonsaponifiable fractions of olive oil and arachis oil also gave some color.

The antimony trichloride color reaction employed by Brockmann and Chen (*3*) has been studied by a number of investigators as the basis for a quantitative method for vitamins D. They used a cold saturated solution of antimony trichloride in dry, alcohol-free chloroform. Both vitamins D_2 and D_3 gave an orange-yellow colored solution with an absorption maximum at 500 mμ. Tachysterol gave a similar reaction, as did cholesterol, sitosterol, ergosterol, 7-dehydrocholesterol, lumisterol, suprasterol I, and suprasterol II. These all gave much weaker color, however, and did not interfere unless present in concentrations over thirty times that of the vitamins D. Vitamin A, with an absorption maximum at 620 mμ, interfered only when present up to six times the concentration of the vitamins D. This reagent thus seemed to serve as a dependable means for the colorimetric determination of vitamins D in the pure state, even when vitamin A and sterols were present in low concentrations.

On the other hand, Emmerie and van Eekelen (*5*) found that all vitamin A must be removed from fish liver oils before the antimony trichloride color reaction is used.

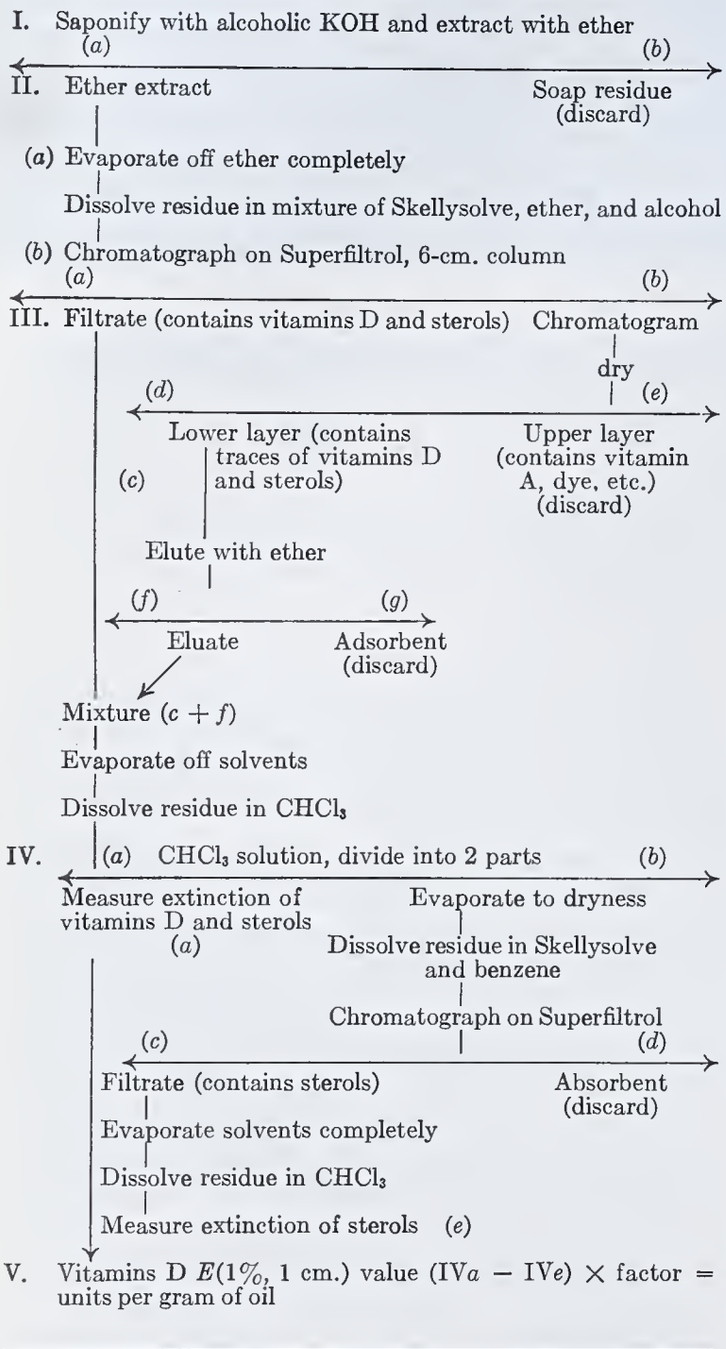
Wolff (*25*) used the Brockmann and Chen color reaction for the determination of vitamin D_2 in nine samples of irradiated ergosterol, taking pure calciferol as the reference standard. His results showed deviations of from 8 to 40 per cent from the bioassay values. He also investigated the determination of vitamins D in fish liver oils, removing the vitamin A and carotenoids by chromatographic adsorption on Montana earth from benzene solution. He found it necessary to remove most of the sterols by the use of digitonin, when the potency of the oil was low.

Ritsert (*17*) removed vitamin A by chromatographic adsorption on aluminum oxide, but reported that this colorimetric method was not applicable to fish liver oils nor to the mixed products obtained by irradiation of the provitamins.

Raoul and Meunier (*15*) modified the reagent of Brockmann and Chen by adding a small amount of acetic anhydride and sulfuric acid to the chloroform solution of antimony trichloride. They attempted to make use of the fading of the vitamin A color and of the concurrent slow formation of a cholesterol color to compute the vitamins D concentration by the rate of change in the absorption maxima. They suggested the use of digitonin to remove most of the sterols.

Nield, Russell, and Zimmerli (*13*) also modified the reagent of Brockmann and Chen by adding acetyl chloride and changing the concentration of the antimony trichloride. Thereby, they increased the sensitivity and permanency of the color.

Milas, Heggie, and Raynolds (*12*) determined the vitamin A potency of fish liver oils by measuring the 620 mμ maximum.



Cholesterol, with an absorption maximum increasing slowly with time, was estimated by measuring the 480 mμ maximum exactly 30 minutes after adding the antimony trichloride reagent to the fish oil sample. Corrections for vitamin A and cholesterol, based on the above measurements, were then applied. Milas *et al.* found better agreement, however, between the calculated potencies and bioassay values when the vitamin A, carotenoids, and possibly 7-dehydrocholesterol were first removed by treatment with maleic anhydride. The vitamins D concentration was then obtained by measurement of the 500 mμ maximum. Gudlet (*7*) also tried the use of maleic anhydride for removing vitamin A and freezing for the removal of sterols.

Ewing and Tomkins (*6*) separated vitamin A from the nonsaponifiable fraction of 16 fish liver oils by chromatographic adsorption on Superfiltrol from hexane-ether-alcohol solution. After removal of sterols with digitonin, the modified reagent of Nield, Russell, and Zimmerli (*13*) was used for the color reaction for vitamins D. Special attention was found necessary in the purification of the chloroform, including a final treatment with activated carbon.

This review indicates that certain interfering substances must be removed from the nonsaponifiable fraction before a quantitative determination of the vitamins D can be made. Then it seems possible to use some modification of the antimony trichloride color method after removal of the vitamin A. The authors chose for removal of the carotenoids, vitamin A, and pigments a modification of the chromatographic adsorption procedure used by Ewing and Tomkins (*6*). Further, while they depended upon freezing and treatment with digitonin for the removal of the sterols, the present authors base their procedure upon a two-step chromatographic treatment where the E(1 per cent, 1 cm.) is determined first for the combined vitamins D and sterols and second for the separated sterols. Then, by difference, the value for the vitamins D is obtained.

The schematic outline of the proposed method is given in stages I to V. The procedure is somewhat complicated; in fact, the minutest details should be followed exactly. To this end, definite instructions are given.

Equipment and Reagents

1. The adsorption columns must be carefully and uniformly prepared to obtain reproducible results. The tubes for the chromatographic determinations are made by sealing a 6-cm. length of 7-mm. Pyrex tubing to the bottom of a 1.6 × 15 cm. (0.625 by 6-inch) Pyrex test tube. These tubes are cleaned before filling by soaking in sulfuric acid–dichromic acid solution, rinsing with distilled water and with alcohol, and finally drying in the flame of a Meker burner. The suction apparatus is designed so that a bank of 8 columns can be developed simultaneously, controlling the pressure with the aid of an open-tube mercury manometer attached to the suction flask. The adsorbent used is a finely divided grade of activated bentonite clay (Superfiltrol, obtained from The Filtrol Corp., 315 West 5th St., Los Angeles, Calif.).

The adsorption columns for the first chromatographic separation are prepared by placing a small wad of cotton in the bottom of one of the adsorption tubes, pressing this down firmly, and adding enough of the clay, so that when very firmly pressed down with a piston, using a cork on the end of a glass rod, under 6 cm. of suction, the height of the packed column will be 3 cm. A second and equal portion of the adsorbent is then added, and pressed down as before to give a hard, level surface. It is important that there should be no air pockets, as they cause irregularly shaped adsorption bands. To aid in overcoming this the piston-head cork used in packing the columns is but slightly smaller than the inside diameter of the Pyrex tube. This also helps avoid loosening of the adsorbent by suction when this cork piston is raised. Other columns for the chromatographic separation of vitamins D and sterols are prepared in the same manner, except that the height is only 1.5 cm. and the filling is done under a suction of 4 cm.

2. Alcoholic potassium hydroxide is prepared by dissolving 14 grams of C. P. potassium hydroxide pellets in 95 per cent ethyl alcohol to give 500 ml. This stock solution is protected from carbon dioxide and filtered through hardened filter paper as needed.

3. C. P. ethyl ether is used without further purification for extracting the saponified oils and for elution of adsorption columns.

4. The anhydrous ethyl ether for the chromatograph is purified by washing C. P. ether with 1 per cent ferrous sulfate solution to remove peroxides, then 10 times with distilled water to remove alcohol, drying with phosphorus pentoxide, filtering, and storing over sodium. This ether is distilled from metallic sodium as needed.

5. The Skellysolve is purified by shaking with concentrated sulfuric acid, washing twice with 10 per cent sodium carbonate solution, then with a mixture of 10 per cent sodium carbonate and 5 per cent potassium permanganate solution. It is washed 15 times with distilled water, the reagent being decanted into a dry flask and dried over sodium. The dried solvent is then distilled (68° to 70° C.) from sodium, the first 5 per cent and the last 10 per cent of the distillate being discarded.

6. The absolute ethyl alcohol is a high-grade commercial product.

7. C. P. chloroform is washed thoroughly with 7 approximately equal portions of water, dried over anhydrous potassium carbonate, decanted, and fractionated, discarding the first and

last 10 per cent of the distillate. This purified chloroform is relatively unstable and hence is prepared in small quantities, and protected from light. Before use, it is tested with silver nitrate solution for chlorides and with potassium iodide and starch solution for oxidizing agents. If these are absent, the chloroform is shaken with activated carbon and filtered just before using.

8. The antimony trichloride reagent is prepared by dissolving 18 grams of C. P. antimony trichloride in the purified chloroform, diluting to 100 ml., filtering, and then adding 2 ml. of redistilled acetyl chloride (*13*). The best results are obtained when not more than one week's supply of the purified chloroform and antimony trichloride reagent is prepared at one time.

9. C. P. thiophene-free benzene is dried over sodium, distilled, and shaken with Superfiltrol before use.

10. Measurements of the absorption maxima at 500 mμ are made on a Bausch & Lomb visual spectrophotometer equipped with a Martins polarizing unit and cells 1 or 2 cm. thick.

Table I. Determination of Conversion Factor[a]

Date Assayed	$E^{1\%}_{1\,cm.}$, 500 mμ	Date Assayed	$E^{1\%}_{1\,cm.}$, 500 mμ	Date Assayed	$E^{1\%}_{1\,cm.}$, 500 mμ
3/21	0.75	3/27	0.80	4/27	0.78
	0.80		0.78		0.78
3/23	0.76	3/31	0.77	5/20	0.76
	0.80		0.76		0.80
3/24	0.74	4/1	0.79	5/22	0.81
	0.75		0.80		0.79
3/25	0.80	4/13	0.74	...	...
	0.82		0.75		...
3/26	0.77	4/14	0.77	...	...
	0.78		0.79		...

Average of 26 determinations $E(1\%, 1\text{ cm.})$, 0.778
Maximum deviation from average, ±5 per cent
Conversion factor $= \frac{15{,}000}{0.778} = 19{,}300$

[a] Using as reference a fish liver oil mixture No. 47,761 having a U. S. P. biological assay value of 15,000 units per gram.

Application of Method

Fish oil samples are weighed in duplicate into 125-ml. Erlenmeyer flasks. Samples containing 4000 to 100,000 U. S. P. units of vitamins D are most convenient. Usually, 0.5 to 2 grams are weighed for natural oils and 0.1 gram for concentrates. If the samples weigh 1 gram or less, 10 ml. of alcoholic potassium hydroxide are added, but for samples weighing more than 1 gram, 10 ml. of alcoholic potassium hydroxide per gram of sample are used. A short-stemmed funnel is then placed in the neck of the flask, to serve as a condenser. The sample is placed in a water bath and kept at 70° to 75° C. for 1 hour, or longer if saponification is not complete. Agitation of the flask at frequent intervals aids this reaction. (At this point the sample may be allowed to stand overnight.)

The sample is cooled to room temperature, 20 ml. of water per 10 ml. of alcoholic potassium hydroxide are added, and it is then extracted in a separatory funnel with four 25-ml. portions of ethyl ether. Gentle shaking in the separatory funnel can be used without danger of emulsification, provided more than the indicated quantity of water has not been added. If an emulsion does form, it can be readily broken by the addition of a few drops of alcohol. The ether layer must always be clear before separation of the two layers.

Finally, all the ether extracts are combined in the separatory funnel and 50 ml. of water are added. When both ether and water layers are clear, the water layer is withdrawn and discarded, leaving the ether layer in the funnel. This preliminary washing, which removes most of the soaps, is repeated twice with fresh 50-ml. portions of water. Agitation during these preliminary washings may result in the formation of a stable emulsion. If this occurs 2 ml. of alcohol are added to break the emulsion. Twenty-five milliliters of water are now added to the ether extract in a separatory funnel and shaken vigorously, after which 25 ml. of water are added and the water and ether layers are allowed to separate. The water layer must be clear before it is withdrawn. This is again repeated twice; at the end of the third washing the ether and the water layers should separate quickly to a sharp interface and appear very clear. The water layer at this point should be colorless and not alkaline to phenolphthalein. If not, the washing must be continued until these conditions are satisfied.

The washed ether extract is now withdrawn into a 125-ml. Erlenmeyer flask, passing through a filter paper containing anhydrous sodium sulfate. The separatory funnel is rinsed with 25 ml. of ether and the rinsings are poured on the filter to remove vitamins D from the sodium sulfate and filter paper. These should be colorless. The ether solution is then evaporated to dryness under reduced pressure, using a water bath at 50° C.

The dry residue from this evaporation is taken up in 5 ml. of a special mixture of solvents prepared from the above purified materials—50 parts of Skellysolve, 10 parts of anhydrous ether, and 1 part of absolute alcohol by volume. One drop of Sudan III solution (25 mg. per liter in the above mixed solvents) is added as a color marker, in the separation of vitamin A and pigments from vitamins D, following the suggestion of Brockmann (*1*) in his isolation of pure vitamin D_3, when he used Sudan III in a 1 to 4 benzene–petroleum ether mixture.

The 6-cm. adsorption column (prepared as previously described) is wetted with 10 ml. of the mixed solvent, and the sample is added, followed by 5 ml. of the solvent for rinsing the flask and 35 ml. of the same mixture for developing the adsorption bands. Each addition of solvent is made just before the top of the adsorption column becomes dry, otherwise it shrinks away from the glass walls and development of the column is not regular. A pressure differential of 6 cm. of mercury is maintained until 35 ml. of the developing solution have been added, when the suction is increased to 10 cm. Further increase of the suction results in crevices in the adsorption column and packing of the adsorbent to such a degree that the flow of the developing solution is nearly stopped.

When the last of the 35 ml. of solution has passed through the column, the adsorbent is dried by drawing air through the column for 5 to 10 minutes. The Pyrex tube is then taken from its suction flask and the top layer of the column is removed to a point 2 mm. below the red Sudan III band, using an L-bent spatula. This removes the vitamin A and pigments. The tube with the remainder of the column (carrying vitamins D and sterols) is replaced in its suction flask and eluted with 25 ml. of ether.

The combined filtrate and eluate are evaporated to dryness under reduced pressure and taken up in 10 ml. of purified chloroform. To 1 ml. of this solution are added 10 ml. of the antimony trichloride reagent. The flask is swirled for 30 seconds, the absorption cell filled, and the extinction determined on the Bausch & Lomb visual spectrophotometer exactly 3 minutes after starting to add the reagent to the vitamin sample. The sample at this point contains vitamins D and sterols.

Table II. Effect of Added Amounts of Sterols to Reference Oil 47,761

(Bioassay, 15,000 U. S. P. units per gram)

Sample No.	Sterol Added	$E^{1\%}_{1\,cm.}$, 500 mμ, 3 Minutes S + D[a]	S	D	Physical-Chemical Method
	Gram				*U. S. P. units/g. (Calcd.)*
Reference oil alone	.....	..	..	0.779	15,035
1	0.010 cholesterol	0.98	0.20	0.78	15,050
2	0.025 cholesterol	1.00	0.23	0.77	14,900
3	0.050 cholesterol	1.01	0.25	0.76	14,700
4	0.100 cholesterol	1.12	0.39	0.73	14,100
5	0.250 cholesterol	1.36	0.64	0.72	13,900
6	0.500 cholesterol	1.84	1.11	0.73	14,100
7	0.001 ergosterol	1.02	0.21	0.81	15,600
8	0.005 ergosterol	0.92	0.12	0.80	15,400

[a] S, sterols.
D, vitamins D.

To correct for the absorption at 500 mμ due to the sterols present, another 1-ml. aliquot of the chloroform solution of vitamins D plus sterols is evaporated to dryness and taken up in 5 ml. of 1 to 2 Skellysolve-benzene mixture. A tightly packed 1.5-cm. Superfiltrol column is wetted with 5 ml. of the mixed solvent and the sample added, followed by the addition of 5 ml. of solvent to rinse the flask and then 50 ml. to elute the sterols. A lavender-blue band carrying the vitamins D is fixed in the upper portion of the adsorption column. The sterols pass into the filtrate. The filtrate is evaporated to dryness under reduced pressure and the

TABLE III. COMPARATIVE VITAMIN D VALUES OF FISH LIVER OILS BY PHYSICAL-CHEMICAL AND BIOLOGICAL METHODS

Sample No.	Physical-Chemical Method: Weight of sample, grams	$E^{1\%}_{1\,cm.}$, 500 mμ	Calcd., U. S. P. units/g.	Biological Method, U. S. P. Units/G.	Difference, %
A. Low Vitamin D Fish Liver Oil Blend					
74,912	2.00	0.14	2,700		
		0.15	2,890	3,000	− 6.8
		Av. 0.145	2,795		
62,321	2.00	0.27	5,210		
		0.25	4,830	4,750	+ 5.7
		Av. 0.260	5,020		
56,211	1.00	0.26	5,020		
		0.27	5,210	4,750	+ 7.7
		Av. 0.265	5,115		
50,261	2.00	0.33	6,370		
		0.31	5,980	6,000	+ 2.9
		Av. 0.320	6,175		
78,992	1.00	0.286	5,520		
		0.242	4,670	4,500	+13.2
		Av. 0.264	5,095		
77,462	1.00	0.43	8,300		
		0.42	8,110	6,300	+30.2
		Av. 0.425	8,205		
66,831	1.00	0.34	6,550		
		0.36	6,850		
		0.36	6,950		
		0.35	6,750	6,600	+ 2.4
		Av. 0.352	6,775		
60,771	2.00	0.38	7,330		
		0.36	6,950	6,600	+ 8.2
		Av. 0.370	7,140		
B. High Vitamin D Fish Liver Oil Blend					
55,691	1.00	0.66	12,700		
		0.65	12,500	12,000	+ 5.0
		Av. 0.655	12,600		
65,251	0.75	0.67	12,900		
		0.68	13,100		
		0.67	12,900		
		0.71	13,700	12,000	+ 9.6
		Av. 0.682	13,150		
65,231	1.00	0.66	12,700		
		0.69	13,300	14,000	− 7.1
		Av. 0.675	13,000		
65,221	0.75	0.70	13,500		
		0.75	14,500		
		0.69	13,300		
		0.67	12,900	14,000	− 3.2
		Av. 0.702	13,550		
41,860	0.50	0.81	15,600		
		0.79	15,200	16,000	− 3.8
		Av. 0.800	15,400		
57,481	1.00	0.75	14,500		
		0.78	15,100	16,500	−10.3
		Av. 0.765	14,800		
40,090	0.50	0.97	18,700		
		0.98	18,900	20,000	− 6.0
		Av. 0.975	18,800		
55,951	0.50	1.49	28,800		
		1.56	30,100	30,000	− 1.8
		Av. 1.525	29,450		
60,761	0.25	1.80	34,700		
		1.89	36,500	35,000	+ 1.7
		Av. 1.845	35,600		
C. Tuna Liver Oils					
68,881	1.00	0.60	11,600		
		0.60	11,600		
		0.62	12,000		
		0.59	11,400	12,000	− 2.9
		Av. 0.602	11,650		
76,902	1.00	0.64	12,400		
		0.67	12,900	12,000	+ 5.4
		Av. 0.655	12,650		
62,071	0.50	0.68	13,100		
		0.66	12,700	16,500	−21.8
		Av. 0.670	12,900		
76,892	1.00	0.797	15,400		
		0.802	15,500	16,000	− 3.4
		Av. 0.800	15,450		
76,882	1.00	1.19	23,000		
		1.15	22,200	21,000	+ 7.6
		Av. 1.170	22,600		
68,871	0.50	1.38	26,600		
		1.27	24,500	25,000	+ 2.2
		Av. 1.322	25,550		
57,951	0.50	1.40	27,000		
		1.38	26,600	28,000	− 4.3
		Av. 1.390	26,800		
D. Tuna Liver Oil Concentrates					
70,202	0.25	3.13	60,400		
		3.22	62,100	65,000	− 5.8
		Av. 3.175	61,250		
63,201	0.10	4.29	82,800		
		4.39	84,700	80,000	+ 4.7
		Av. 4.340	83,750		
70,712	0.10	3.44	66,400		
		3.56	68,700		
		3.50	67,600		
		3.50	67,600	80,000	−15.6
		Av. 3.500	67,570		
49,831	0.10	7.80	151,000		
		7.69	148,400	160,000	− 6.4
		Av. 7.750	149,700		
44,470	0.10	12.30	237,400		
		11.9	229,700	240,000	− 2.7
		Av. 12.100	233,550		
57,381	0.10	12.5	241,300		
		12.3	237,400	300,300	−20.2
		Av. 12.4	239,350		
61,751	0.10	15.2	293,400		
		14.9	288,000	325,000	−11.9
		Av. 15.05	290,500		
E. Vitamins D Fish Liver Oil Distillates					
55,031	1.00	0.96	18,500		
		0.93	17,900	18,500	− 1.6
		Av. 0.945	18,200		
44,170	0.25	0.84	16,200		
		0.76	14,700	15,000	+ 3.0
		Av. 0.800	15,450		
56,961	1.00	1.11	21,400		
		1.09	21,000	20,000	+ 6.0
		Av. 1.100	21,200		
44,090	0.50	1.12	21,600		
		1.13	21,800	22,000	− 1.4
		Av. 1.125	21,700		
43,040	0.25	1.68	32,400		
		1.69	32,600	30,000	+ 8.3
		Av. 1.685	32,500		
44,080	0.25	1.56	30,100		
		1.52	29,300	31,000	− 4.2
		Av. 1.540	29,700		
F. Miscellaneous Vitamins D Fish Liver Oils					
85,682 Cod	4.10	0.0124	239		
		0.0165	318	250	+11.2
		Av. 0.0144	278		
62,151 Cod	5.00	0.008	154		
		0.0102	197	287	−39.4
		Av. 0.009	174		
62,331 Cod	4.10	0.0091	186		
		0.0096	186	300	−38.0
		Av. 0.0094	186		
52,051 Halibut	0.40	0.066	1,270	1,200	+ 5.8
62,171 Halibut	2.00	0.099	1,910		
		0.094	1,810	1,200	+55.0
		Av. 0.097	1,860		
57,991 Yellow Fin	1.00	0.153	2,950		
		0.162	3,130	3,000	+ 1.3
		Av. 0.158	3,040		
80,352 Swordfish	1.00	0.37	7,140		
		0.34	6,560	5,000	+37.0
		Av. 0.355	6,850		
80,362 Swordfish	1.00	0.48	9,260		
		0.43	8,300	10,000	−12.2
		Av. 0.455	8,780		
57,971 Albacore	0.25	3.03	58,500		
		2.94	56,700	55,000	+ 4.7
		Av. 2.985	57,600		
58,011 Bonita	0.25	3.11	60,000		
		3.33	64,300	65,000	− 4.4
		Av. 3.220	62,150		
75,442x[a] Outside oil	0.50	2.33	45,000		
		2.42	46,700		
		2.46	47,500		
		2.37	45,700	50,000	− 7.6
		Av. 2.40	46,225		
B2861[a] Outside oil	0.50	1.43	27,600		
		1.44	27,800	32,500	−14.1
		Av. 1.44	27,700		
P6846x[b] Outside oil	1.00	1.05	20,300		
		1.02	19,700	20,000	0.0
		Av. 1.04	20,000		
V3428[b] Outside oil	0.10	61.5	1,187,000		
		65.4	1,260,000	1,200,000	+ 2.0
		Av. 63.5	1,223,500		

[a] Sample and biological data supplied by S. H. Fox, Gelatin Products, Inc.
[b] Sample and biological data supplied by A. E. Briod, National Oil Products Co.

residue taken up in 1 ml. of chloroform. To this are added 10 ml. of the antimony trichloride reagent, using a 2-cm. absorption cell. The extinction is determined at 500 mμ exactly 3 minutes after mixing.

From these two extinction values, the E(1 per cent, 1 cm.) is calculated for vitamins D and sterols combined and for sterols alone. The difference between these two values gives the E (1 per cent, 1 cm.) for the vitamins D in the original sample. This value, multiplied by the factor 19,300 referred to below, gives the potency in U. S. P. units per gram of oil.

Reference Oil. A typical vitamins D mixed fish liver oil, No. 47,761, was selected as the reference oil and was bioassayed several times by the official U. S. P. method, giving an average potency of 15,000 U. S. P. units per gram. This oil was also carefully assayed 26 times by the proposed chemical method over a period of 2 months. The extinctions are given in Table I.

During this period several new lots of adsorbent, solvents, and antimony trichloride were used. The average E(1 per cent, 1 cm.) value is 0.778 with a maximum deviation of only ±5 per cent. With this figure and the biological potency value of 15,000 units per gram, the standard conversion factor is calculated as 19,300 and is used throughout this report.

As a further check on the method, it was decided to determine whether cholesterol and ergosterol would interfere with the actual analysis of a fish liver oil. Accordingly, varying amounts of these substances were added to 1-gram samples of the reference oil (No. 47,761) and analyzed for vitamins D (Table II). These data show that adding even 0.5 gram of cholesterol above that already present in the natural fish liver oil caused an error of less than 7.3 per cent in the analysis. This amount of cholesterol is far higher than would be found in a fish liver oil. For 0.050 gram of added cholesterol the error due to the added cholesterol is only 2 per cent and this is well within the limit of reproducibility of the method as shown by Table I.

Further, the presence of the provitamin ergosterol (Table II, 7, 8), in concentrations of slightly higher order than the vitamin itself, caused only an insignificant increase in the calculated potency, indicating that no measurable amount of the vitamins D was formed during the analytical procedure.

Experimental Results

The application of the method was carried out on the following types of fish liver oil (Table III):

		Units per gram
A	Low vitamin D oil blends	3,000 to 7,000
B	High vitamin D oil blends	12,000 to 35,000
C	Tuna liver oils	12,000 to 28,000
D	Tuna liver oil concentrates	65,000 to 325,000
E	Vitamins D liver oil distillates	15,000 to 31,000
F	Miscellaneous fish liver oils	250 to 1,200,000

The results in Table III are self-explanatory, and show that the method is capable of reproducibility if the details of the procedure are followed exactly. The chemical determinations were made in duplicate or triplicate—that is, two or more samples were weighed out and carried through the entire procedure.

The over-all inspection of the results indicates a fairly close agreement between the physical-chemical method and the official U. S. P. procedure. The most outstanding variance lies in miscellaneous vitamins D fish liver oils, where it seems that with oils of a potency of 1200 or less U. S. P. units per gram, the errors are large. This may perhaps be attributed chiefly to taking large samples for test. This point is brought out clearly in the case of halibut oils 52,051 and 62,171. The outstanding differences may be due in part to a combination of errors, including those in the bioassay values which in themselves have a range of at least ±10 per cent. The average percentage difference for all 51 oils is 9.7, and if we exclude the 7 oils which show a range of 20 per cent or more, the average for the remaining 44 samples is 5.8 per cent.

The last four oils listed in Table III were submitted as unknowns, for a further check on the efficiency of the chemical method. The biological assays were reported as having been carried out by the official U. S. P. method. The results agree satisfactorily.

Summary and Conclusions

A physical-chemical method for determining vitamins D in fish liver oils is based upon the separation of the vitamins D from vitamin A and other interfering substances by chromatograph adsorption, and then measurement of the extinction coefficient at 500 mμ of the reaction product in antimony trichloride.

Data are presented for the vitamins D potency of 51 liver oils from various types of salt-water fish. The values indicate that the proposed physical-chemical method, as outlined, gives results which are in fairly close agreement with those by the U. S. P. procedure for oils ranging from 5000 units per gram and up in potency. For weaker oils, the method is not so satisfactory. Both possible simplification and its application to irradiated products are being studied.

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Pantothenic Acid

Optical Rotation As a Measure of Stability

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STABILITY studies in this laboratory on synthetic and naturally occurring pantothenic acid have indicated a high degree of lability for the compound in presence of acid or alkali. The rate of destruction in preliminary studies appeared as a direct function of pH and of temperature. Furthermore, the presence of certain compounds in solution catalyzed the destruction of pantothenic acid even in the pH range of optimum stability.

A rapid chemical or physical method of analysis was desired to follow the rate of destruction of pantothenic acid and its salts under various controlled conditions. Since the nature of the pantothenate molecule made it unlikely that chemical derivatives of specific analytical value could be found, the author turned to physical methods of analysis. The optical rotatory power of the compound appeared as one possibility, but presented some difficulties, since the nature of the destruction was not entirely known and at least one of the known hydrolysis products was also optically active. Only the dextrorotatory form of pantothenic acid is biologically active and the study was limited to this form.

Evidence of the structure of pantothenic acid first arose from a knowledge of its normal degradation products, and this in turn provided clues to the various methods of synthesis.

Williams, Weinstock, Rohrmann, Truesdail, Mitchell, and Meyer (*6*) in 1939 indicated that pantothenic acid undergoes cleavage in acid or alkaline media to form β-alanine and an aliphatic dihydroxy acid. Woolley, Waisman, and Elvehjem (*7*) confirmed this finding by condensing the acid halide of the dihydroxy acid fragment of pantothenic acid with β-alanine to regenerate a compound having the full biological action of the original compound. Weinstock, Mitchell, Pratt, and Williams (*4*) isolated β-alanine as one of the cleavage products shortly thereafter, and Woolley, Waisman, and Elvehjem (*8*) made the further observation that the dihydroxy acid portion of the molecule readily forms a lactone. The dihydroxy acid portion of the molecule was later determined as α,γ-dihydroxy-β,β-dimethyl butyric acid by Williams and Major (*5*). Various synthetic methods have since appeared from many laboratories which depend on the condensation of the dihydroxy acid, or its lactone or salt, with β-alanine to form pantothenic acid.

Theory

Simple hydrolytic cleavage (I) of the pantothenate ion to yield β-alanine and *d*(+)-α,γ-dihydroxy-β,β-dimethylbutyric acid [hereinafter designated as *d*(+)-dihydroxy acid] and lactonization (II) of the *d*(+)-dihydroxy acid to form *d*(−)-α-hydroxy-β,β-dimethylbutyrolactone [hereinafter designated as *d*(−)-lactone] are pictured as in the formula to the right.

A 1 per cent solution of calcium *d*(+)-pantothenate theoretically gives rise to a 0.545 per cent solution of the *d*(−)-lactone, assuming complete hydrolysis of pantothenate and complete lactonization of the *d*(+)-dihydroxy acid. The rate and completeness of lactonization were not known; however, it appeared likely that rotation values could be established for known systems of the *d*(−)-lactone-*d*(+)-dihydroxy acid equilibrium which would be of value in predicting the nature of unknown systems which also contained unchanged pantothenate.

Experiments were carried out to determine whether or not the above reactions could be reduced to a mathematical basis in which the measured rotation would serve as an index to the amount of unchanged pantothenate ion in solution at any time.

Experimental

The specific rotation of several samples of synthetic calcium *d*(+)-pantothenate in 1 per cent aqueous solution ranged from +25.5° to +27°. The values for highly purified *d*(−)-α-hydroxy-β,β-dimethylbutyrolactone ranged from −50° to −51.5°. [Grüssner, Gätzi-Fichter, and Reichstein (*1*) reported the $[\alpha]_D^{17.5}$ of the *d*(−)-lactone to be −49° (C = 4.012 in water) and the $[\alpha]_D^{19}$ of the barium salt of the corresponding acid to be +5.5° (C = 2.8 in water).] All readings were taken at 25° to 26° C., using a sodium arc lamp, a 1-decimeter tube, and a rotary polariscope with Lippich polarimeter.

From the above-determined values a freshly made solution containing 2 parts of calcium *d*(+)-pantothenate to 1 part of *d*(−)-lactone would be expected to display zero rotation, since in these proportions the opposite rotatory powers of the two compounds almost exactly cancel out. This point was proved by experiment. A 0.545 per cent solution of the *d*(−)-lactone was found to have the calculated rotation, −27.5°. In 0.1 N sodium hydroxide the rotation shifted to +7 as the lactone ring opened to give the *d*(+)-dihydroxy acid. This change was quantitatively reversed by making the solution strongly acid. The reaction rate in either direction was greatly accelerated by heat.

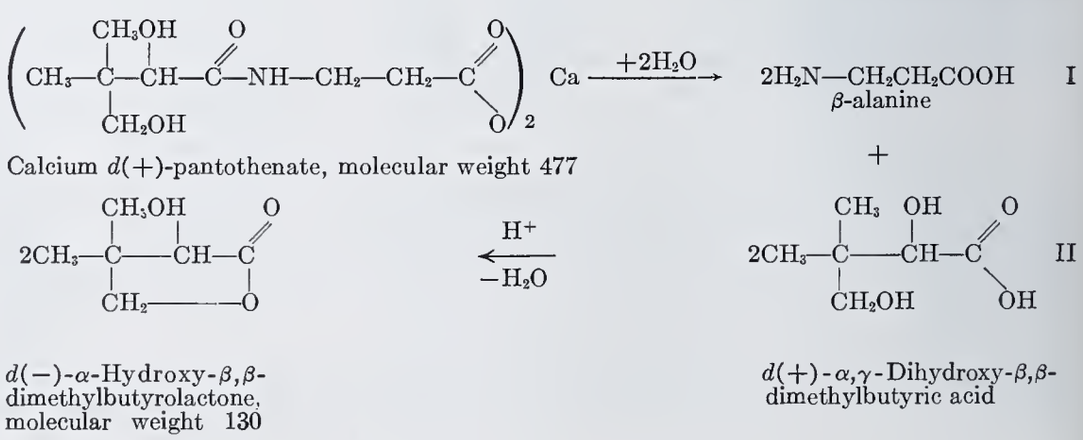
The changes in optical rotation of a 1 per cent solution of calcium *d*(+)-pantothenate as it undergoes hydrolysis and lactonization of the *d*(+)-dihydroxy acid are pictured in two steps as follows:

Ca *d*(+)-pantothenate → *d*(+)-dihydroxy acid $\xrightarrow{H^+}$ *d*(−)-lactone

$[\alpha]_D^{25} = +27°$ $\quad [\alpha]_D^{25} = +7°$ $\quad [\alpha]_D^{25} = -27.5°$

C = 1 per cent $\quad C$ = 0.545 per cent

Ideal curves representing the rotation through all stages of cleavage of calcium pantothenate, assuming zero (curve I)



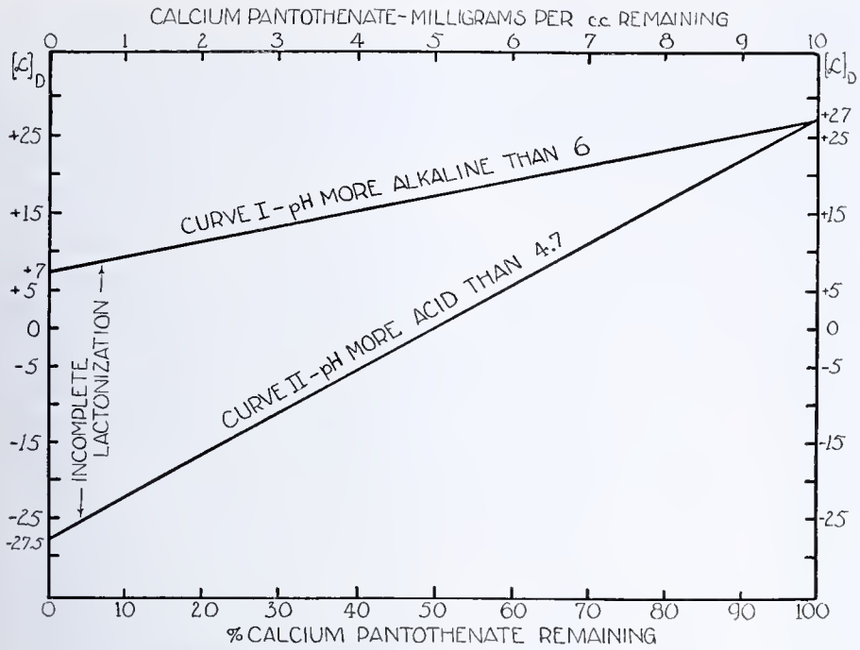


FIGURE 1. SPECIFIC ROTATION OF 1 PER CENT CALCIUM *d* (+)-PANTOTHENATE

Representing zero to complete hydrolysis at different pH levels

and complete (curve II) lactonization of the *d*(+)-dihydroxy acid, are shown in Figure 1.

The validity of curve I was tested by subjecting 1 per cent solutions of calcium pantothenate to treatment with 0.01 *N* sodium hydroxide at various temperatures and taking the rotation at intervals. In all instances the specific rotation finally shifted from +27° to +7°. The reaction required several weeks at room temperature, but only about 24 hours at 100° C.

In order to test the validity of curve II, the following experiment was conducted, in which unbuffered solutions of calcium *d*(+)-pantothenate were made to acid pH to favor lactonization of the *d*(+)-dihydroxy acid.

CALCIUM PANTOTHENATE UNBUFFERED. One per cent solutions of calcium *d*(+)-pantothenate were made to pH 4.0, 4.6, and 5.2 with hydrochloric acid and placed in well-capped bottles in a 60° oven. The rate of destruction was followed at 5-day intervals by measurement of the rotation and by microbiological assay by the method of Strong, Feeney, and Earle (*2*). The general good agreement between the values estimated from curve I and the microbiological assay values is shown in Table I.

TABLE I. ESTIMATION OF RATE OF DESTRUCTION OF CALCIUM *d*(+)-PANTOTHENATE AT 60° C. IN UNBUFFERED SOLUTION BY MEASURE OF OPTICAL ROTATION

Sample	Treatment °C.	Treatment Days	pH	$[\alpha]^{25}_D$ (C = 1%)	Calcium Pantothenate, Per Cent of Original: Estimated	Calcium Pantothenate, Per Cent of Original: Microbial assay
A	Original		4.0	+27	100	100
	60	5	4.3	+11	71	73
	60	10	4.6	+ 7	63.5	66
	60	15	4.8	+ 3	56	55.5
	60	20	5.0	− 1	48	45.6
B	Original		4.6	+27	100	100
	60	5	4.8	+18.5	85	84
	60	10	5.1	+17	82	81
	60	15	5.3	+14	76.5	81
	60	20	5.5	+12	71	68
C	Original		5.2	+27	100	100
	60	15	5.9	+27	100	100
	60	20	5.9	+27	100	95

The gradual rise in the pH of all solutions can be ascribed to the liberation of β-alanine, which has an isoelectric point close to pH 6, and to the gradual lactonization of the *d*(+)-dihydroxy acid, which exerts a pH effect about 3.5 as the free acid. The rate of pH change decreased as the more stable range was approached. This paralleled the lessened rate of destruction.

EFFECT OF pH ON *d*(−)-α-HYDROXY-β,β-DIMETHYLBUTYROLACTONE. The above experiment indicated that lactone formation was complete below pH 5.0, as might be expected. In order to determine the effect of pH on the extent of lactonization, the following experiments were conducted:

A 1 per cent solution of the *d*(−)-lactone was subjected to 60° for 10 days. The original pH was 4.6 and had changed to 3.6 at the end of the period; however, the original specific rotation of −51° did not change significantly throughout the period.

The necessity of buffering solutions for further study was indicated, and equal aliquots of a 1 per cent solution of the *d*(−)-lactone were made to pH 7.4, 6.6, 6.0, and 4.8 with mixtures of 3 per cent solutions of monosodium and disodium phosphate. Original rotations were taken immediately. The solutions were placed at 60° and samples were removed for rotation measurements in 2, 5, and 7 days (Table II). Equilibrium between the lactone and acid forms was apparently reached in about 2 days and was not changed significantly thereafter.

TABLE II. EFFECT OF pH ON SPECIFIC ROTATION OF *d*(−)-α-HYDROXY-β,β-DIMETHYLBUTYROLACTONE AT 60° C.

(C = 0.545 per cent)

	pH	$[\alpha]^{25}_D$	pH	$[\alpha]^{25}_D$	pH	$[\alpha]^{25}_D$	pH	$[\alpha]^{25}_D$
Original	7.6	−28	6.6	−27	6.0	−27	4.8	−27
60° C., 2 days	7.2	+ 7	6.4	− 1	5.8	− 9	4.6	−26
60° C., 5 days	7.2	+ 6	6.4	+ 1	5.8	− 8	4.6	−27
60° C., 7 days	7.2	+ 6	6.4	+ 2	5.8	− 7	4.6	−25
60° C., 14 days	7.2	+ 6	6.4	+ 2	5.8	− 6	4.5	−25

In view of the above finding, it appeared possible to calculate corrections from the standard decomposition curve of pantothenic acid to account for incomplete lactone formation at any constant pH. The following experiment was run to test this possibility:

CALCIUM PANTOTHENATE BUFFERED, CORRECTION FOR INCOMPLETE LACTONIZATION. A 1 per cent solution of calcium *d*(+)-pantothenate and a 0.545 per cent solution of the *d*(−)-lactone were made up and each solution was divided into four equal parts. The equal parts of the two solutions were then paired off and the members of each pair were made to comparable pH, using small amounts of monosodium and disodium phosphate, sodium hydroxide, and concentrated phosphoric acid as required. Calcium phosphate precipitated in the calcium pantothenate solutions, but did not interfere with the collection of data. All solutions were kept at 60° and aliquots were taken for rotation and assay at 5-day intervals. Small pH changes occurred throughout the experiment, but the pH in each case was maintained in a narrow range. The pH ranges for the paired solutions are shown in Table III.

The *d*(−)-lactone was stable at pH 3.9 to 4.7. In the range pH 4.9 to 5.2, the hydrolysis of the lactone occurred very slowly and to only a slight extent. At pH 5.6 to 5.9 the

Table III. Estimation of Rate of Destruction of Calcium $d(+)$-Pantothenate at 60° C. in Buffered Solution

[By measure of optical rotation and correction for paired solutions of $d(-)$-lactone]

Group Pair	Sample	$[\alpha]_D^{25}$ Lactone (C = 0.545%)	$[\alpha]_D^{25}$ Ca Pantothenate (C = 1%)	Ca Pantothenate, Per Cent of Original: Estimated Uncorrected	Estimated Corrected	Microbial assay, found
	Days					
I	Original	−27.5	+27	100	.....	100
pH 3.9–4.2	1	−27	+22	91	.....	90
	5	−27.5	+12	73	.....	...
	15	−27.5	−16	21	.....	23.3
	19	−27.5	−20.5	13	.....	13.1
	25	−27.5	−24	6	.....	6.3
II	Original	−27.5	+26	100	.....	100
pH 4.4–4.7	1	−27	+16	80	.....	80.5
	5	−27	+ 7	63	.....	69
	15	−27	−11	29	.....	27
	19	−27.5	−14	25	.....	21.9
	25	−27.5	−19	14	.....	14.4
III	Original	−27.5	+26	100	.....	100
pH 4.9–5.2	1	−27.5	+21	89	.....	89
	5	−27.5	+13	74	.....	78
	15	−25	+ 3	55	53	52.7
	19	−25	+ 1.5	53	51	58.4
	25	−23	− 3	44	41	38.3
IV	Original	−27.5	+27	100	100	100
pH 5.6–5.9	1	−23	+25	97	96	96
	5	−22	+23	93	92	95
	15	−16.5	+19.5	87	84	76.6
	19	−16	+10	69	61.5	63.6
	25	−15	+11	70	62.5	61.8

hydrolysis of the lactone was more rapid in the initial stages but continued throughout the entire period. No correction appeared to be necessary in the calcium pantothenate estimations in groups 1 and 2, and curve I (Figure 1) was applied with fair success, as seen in Table III. Corrections for incomplete lactone formation were made, as indicated, for groups 3 and 4. The corrections were made by varying the standard curve (Figure 1) to correspond with the specific rotation value obtained for the $d(-)$-lactone at the particular pH and time in question. These corrections are rapidly obtained for paired solutions by extending a line on the standard curve from the +27° point for 1 per cent calcium pantothenate to the $d(-)$-lactone value in question and reading the residual calcium pantothenate of the paired pantothenate solution directly from its rotation value. All the correction curves fall naturally between curves I and II (Figure 1). As seen in Table III, the corrected estimates are in somewhat better agreement with the microbiological assay values than the uncorrected estimates.

Effect of pH on Lactonization of $d(+)$-α,γ-Dihydroxy-β,β-dimethylbutyric Acid. In the foregoing experiments the equilibria values obtained at various pH values starting from the $d(-)$-lactone were thought to be equivalent to those which would be obtained starting from the corresponding $d(+)$-dihydroxy acid. That this assumption is essentially correct appears to be borne out by the results of the experiments; however, a large difference may exist in the rate of closing and opening of the lactone ring, and the effect of temperature on the equilibrium between the two forms obtained at any constant pH was not known. The following experiments were run to study the effect of pH and temperature on the rate and completeness of lactonization of the $d(+)$-dihydroxy acid.

A 0.545 per cent solution of the $d(-)$-lactone in 0.01 N sodium hydroxide was converted quantitatively to the $d(+)$-dihydroxy acid by mild heating. The solution was divided into 5 equal parts which were made to varying pH values by adding appropriate mixtures of dry phosphate buffer plus hydrochloric acid as needed (Table IV). Half of each buffered solution was placed at 60° and the other half maintained at room temperature. Optical rotation measurements were made at intervals, as shown in Table IV.

Lactonization proceeded much more rapidly at 60° than at room temperature. Equilibrium was attained between the acid and lactone forms at each pH level in a period of less than 20 days. This was true also in the preceding experiments (Tables II and III) when starting from the $d(-)$-lactone.

Room-temperature studies with the $d(+)$-dihydroxy acid (Table IV) indicated that equilibria values, particularly at the higher pH levels, may be different from those obtained at 60°. Equilibrium was not reached in 55 days in the samples at lower pH levels.

The rate of lactonization of $d(+)$-dihydroxy acid both at 60° and at room temperature appears to be greater than the rate of hydrolysis of pantothenate at all pH levels where the pH favors lactonization. Thus there should be no large excess of $d(+)$-dihydroxy acid arising from hydrolysis of pantothenate in solution at any time at pH levels which favor complete lactonization. At pH levels where lactonization is partial—i. e., pH 4.7 to 6—correction has been made most satisfactorily by use of paired solutions of the $d(-)$-lactone, as previously described. Use of the $d(+)$-dihydroxy acid has not proved so satisfactory for paired solutions at 60°.

As can be seen by comparing Tables III and IV, equilibria values at equal pH levels at 60° are about the same whether starting from the $d(-)$-lactone or the $d(+)$-dihydroxy acid. Thus, when equilibrium is reached, the correction will be about equal starting from either compound. Further study is needed to establish the validity of the method of correction as herein applied, particularly in regard to the beginning stages of pantothenate destruction.

Table IV. Effect of pH on Lactonization of $d(+)$-α,γ-Dihydroxy-β,β-dimethylbutyric Acid at Room Temperature and at 60°

[C = 0.545%, as $d(-)$-α-hydroxy-β,β-dimethylbutyrolactone]

Time of Sampling	pH 3.9–4.2	pH 4.4–4.7	pH 4.9–5.2	pH 5.3–5.6	pH 6.0–7.0
Days			$[\alpha]_D^{25}$		
			Samples Maintained at 60°		
Original	+ 7	+ 7	+ 7	+ 7	+ 7
1	− 6	+ 1	+ 3	+ 3	+ 5
4	−13	− 7	− 4	0	+ 4
8	−22	−17	−11	− 6	+ 4
12	−26	−25	−15	−12	+ 4
20	−28	−27	−23	−17	+ 4
35	−28	−27	−23	−17	+ 4
			Paired Room Temperature Samples		
Original	+ 7	+ 7	+ 7	+ 7	+ 7
1	+ 2	+ 6	+ 6	+ 7	+ 7
4	0	+ 4	+ 7	+ 7	+ 7
8	− 3	+ 1	+ 5	+ 7	+ 7
12	− 8	0	+ 3	+ 5	+ 7
20	−11	− 3	0	+ 3	+ 7
35	−17	− 7	− 2	+ 3	+ 7
55	−24	−11	− 5	+ 3	+ 7

Effect of Excess Acid on Rotation and Rate of Hydrolysis of Calcium $d(+)$-Pantothenate. One per cent solutions of calcium $d(+)$-pantothenate, containing 0.5 per cent of chlorobutanol as a preservative, were made to pH

2.2 and 1.3 with concentrated hydrochloric acid. Twice as much was used to acidify to pH 1.3 as was used to acidify to pH 2.2. The initial specific rotation of these solutions was +22° in each case. Further experiments indicated that the specific rotation of pantothenate is decreased progressively at pH more acid than 4. Stability studies were conducted on the above solutions with a control solution at pH 6.3, as shown in Table V.

In the case of solutions more acid than pH 4, estimations for residual pantothenate made from curve II (Figure 1) fell considerably below the values found by microbiological assay. This followed naturally from the fact that the specific rotation of calcium pantothenate was depressed from +27° to +22° at this pH. Experiments showed that the rotation of *d*(−)-lactone was not changed at pH more acid than 4. A curve then drawn from +22° to −27.5° gave values in good agreement with those found by microbiological assay (Table V).

Table V. Specific Rotation of Calcium *d*(+)-Pantothenate at Room Temperature in Presence of Excess Acid

Sample	pH	$[\alpha]^{25}_{D}$ (C = 1%)	Estimated	Microbial Assay, Found
Days			%	%
Original	1.3	+22	100	.....
1	1.3	+20.5	97	95
8	1.4	+ 6	67.5	67.2
20	1.5	− 3	49	48.9
40	1.7	−12	30.5	27.3
Original	2.2	+22	100	.....
1	2.0	+20	96	93.7
8	2.1	+16	88	86
20	2.26	+14	83	79.4
40	2.3	+ 8	71	68
Original	6.3	+27	100	.....
1	6.2	+27	100	.....
8	6.2	+27	100	97.5
20	6.5	+27	100	99.8
40	6.9	+28	100	98.2

Application of Method

Under optimum conditions the presence of thiamine, riboflavin, nicotinamide, pyridoxine, or various combinations did not obviate application of the method for rapid study of stability of pantothenate. In the pH range in which it is most stable—i. e., more acid than 6.6—riboflavin has no appreciable optical rotatory power. None of the other compounds mentioned is optically active.

These studies clearly demonstrate the incompatibility of thiamine and pantothenate in aqueous mixtures. Pantothenate is most stable in a pH range of about 5.5 to 7. Hydrolysis of the molecule occurs at an increasing rate as the pH moves away from this range on either the acid or alkaline side. Thiamine becomes increasingly less stable at pH more alkaline than 4, as shown by a large number of independent experiments conducted in this laboratory during the last four years.

In presence of air, pyridoxine is oxidized to a colored compound of sufficient intensity to obviate rotation measurements. When oxygen was excluded pyridoxine was found to be compatible with pantothenate at neutral pH.

Nicotinamide in concentrations from 2 to 15 per cent had a stabilizing effect for pantothenate at pH 7 to 9, but not at pH 4 to 5 where it appeared to have a small labilizing effect. The effect of thiamine and riboflavin on pantothenate was negligible.

Since hydrolysis appeared as the main mechanism of destruction of pantothenate, it seemed likely that the molecule would resist dry heating up to the point of decomposition.

To test this hypothesis, dry calcium pantothenate was heated at 120° for 40 hours. Rotation measurements on the heat-treated pantothenate indicated about 12 per cent destruction. Since the presence of traces of moisture might account for considerable destruction of pantothenate, a similar experiment was run in which the calcium pantothenate was mixed with an equal weight of a dehydrating agent—i. e., silica gel or calcium sulfate—preliminary to heating. In this experiment no destruction of pantothenate occurred, as measured by both the rotation method and the microbiological assay method. Further evidence that traces of water may react to destroy pantothenate at high temperatures was obtained by mixing calcium pantothenate with an equal weight of disodium hydrogen phosphate dodecahydrate and heat-treating the mixture as above. About one third of the pantothenate was destroyed by this treatment. In presence of anhydrous disodium phosphate only 2 to 4 per cent of the original pantothenate was lost.

Acidic substances appeared to exert a catalytic destructive effect toward pantothenate even when only traces of moisture were present. Thus, addition of small amounts of benzoic acid, succinic acid, or nicotinamide hydrochloride caused rapid destruction of admixed calcium pantothenate at 120°.

Calcium pantothenate is moderately hygroscopic and might be expected to be somewhat less stable in a humid than in a dry atmosphere. Calcium pantothenate samples alone and admixed with equal weights of silica gel and calcium sulfate were placed in a container together with a vessel from which water could evaporate freely and the whole was subjected to 60° for 2 weeks. No destruction of pantothenate in presence of either desiccant took place as shown by rotation measurement. Destruction of pantothenate in absence of a desiccant was slight but detectable and was estimated at about 5 per cent of the original.

Discussion

The suggested method for estimation of pantothenate in solutions of known chemical content deserves further study both to establish the validity of the method and to increase its precision. The microbiological assay method of Strong, Feeney, and Earle (*2*) is thought to be accurate to within ±5 per cent when no interfering substances are present. In most instances in this study, values for pantothenate estimated by rotation were within ±5 per cent of those found by the microbiological method. The rotation method is thought to be precise for simple mixtures at acid pH where lactone formation parallels the rate of destruction, and is applicable, though inherently less accurate, at neutral or alkaline pH where no lactone formation occurs. At intermediate pH values where correction for incomplete lactone formation is needed—i. e., pH 4.5 to 6—the accuracy of the method is largely dependent on the accuracy of the correction.

The pH of optimal stability of pantothenate is in the range of pH 5.5 to 7, but may vary somewhat, depending on the presence of materials which may catalyze hydrolysis. The presence of phosphate buffer appeared to catalyze hydrolysis of pantothenate (compare Tables I and III). This observation was confirmed by further studies in which the microbiological assay was used exclusively. The presence of electrolytes in general appeared to have slight catalytic effect toward destruction of pantothenate. Stability was not so satisfactory in presence of other β-complex vitamins at neutral or acid pH as in their absence at similar pH. Pantothenate in water alone appeared to be stable for 20 days at 60° at pH 5.5 to 7.

Susceptibility of pantothenate to hydrolytic cleavage in the semidry form where only small amounts of moisture are concerned was demonstrated in various ways. The rate of destruction, just as in solution, was dependent upon effective pH. Methods of food drying and processing can be expected to have a large effect on the amount of pantothenate destroyed. Rapid and complete drying and storage in the cold should yield by far the best results. Destruction of pantothenate in natural grain rations for the chick has been reported (*3*) to occur in 100 hours at 100° or more completely in 30 hours at 120°. The amount of water derived from the

feed mixture is apparently sufficient under these conditions to allow cleavage of the pantothenate contained therein. This appears as the most likely explanation, since pantothenate was found completely stable under even more drastic conditions when kept entirely free from moisture.

Unfortunately, the natural stability of many of the vitamins—i. e., thiamine, pyridoxine, and ascorbic acid—is poor at the pH of optimal stability of pantothenate. This fact deserves careful consideration in the case of pharmaceutical preparations and foods where loss of pantothenate is to be avoided.

Summary

Pantothenate destruction under ordinary conditions can be traced to hydrolysis of the molecule. A method is described for following the destruction of calcium $d(+)$-pantothenate by rapid polarimetric analysis.

The rate of pantothenate destruction is a function of pH and temperature and is affected also by presence of other substances both in aqueous solution and in dry mixtures. Optimum stability of pantothenate lies in the approximate range, pH 5.5 to 7. The rate of destruction increases as the pH moves away from this range. Only traces of water are needed to cause significant destruction of pantothenate when other conditions favor hydrolysis.

Special significance is attached to the apparent incompatibility of pantothenate with certain other vitamins, notably thiamine.

Acknowledgment

Acknowledgment is gratefully made to Eleanor Willerton, who conducted the microbiological assays. Thanks are expressed to Edmond E. Moore and Marjorie B. Moore for helpful discussion of many of the problems involved and for a supply of $d(-)$-α-hydroxy-β,β-dimethylbutyrolactone, and to Carl Nielsen and E. H. Volwiler for helpful advice and support of this project.

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Colorimetric Determination of Cobalt with *o*-Nitrosoresorcinol

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YOE and Barton (*2*) reported a study of the reaction of β-nitroso-α-naphthol with cobalt, including spectrophotometric data, and found the reaction applicable to the colorimetric determination of small amounts of cobalt. The main disadvantage in using this reagent is due to the insolubility of the cobalt complex. The colored suspension tends to precipitate on standing, resulting in a limited stability.

The authors observed that a similar organic compound, *o*-nitrosoresorcinol, also reacts with cobalt and may be advantageously employed as a colorimetric reagent for this element. *o*-Nitrosoresorcinol is slightly less sensitive for cobalt than is β-nitroso-α-naphthol, but solutions of the cobalt complex of *o*-nitrosoresorcinol are stable for several weeks.

Cronheim (*1*) used *o*-nitrosophenol for the colorimetric determination of cobalt, extracting the cobalt complex with petroleum ether and measuring the intensity of the colored ether fraction. The authors were unable to extract the cobalt complex of *o*-nitrosoresorcinol with any immiscible solvent.

Spectrophotometric data for solutions of *o*-nitrosoresorcinol, the cobalt complex, and the complexes of other metals are presented in this paper. A colorimetric method for the determination of cobalt in the presence of nickel is given.

Apparatus and Materials

Transmittancy measurements were made with a Beckman spectrophotometer, Model D, using a solution thickness of 1 cm. and distilled water as a standard.

The visual observations were performed with 50-ml. (220-mm.) Nessler tubes.

pH measurements were made with the glass electrode.

o-Nitrosoresorcinol (Eastman No. 2088). An aqueous 0.05 per cent solution of the sodium salt was employed. This reagent solution is stable for several weeks.

Cobalt. A stock solution containing 1 mg. of cobalt per ml. was prepared from c. p. cobalt nitrate hexahydrate. Solutions containing 20 or 100 p. p. m. of cobalt, prepared by dilution of the stock solution, were used in the experiments.

Nickel. c. p. nickel nitrate hexahydrate was purified by precipitating, as potassium cobaltinitrite, any cobalt present and filtering. The nickel was precipitated as the hydroxide, filtered, washed thoroughly, and dissolved in hydrochloric acid. The nickel content was determined gravimetrically with dimethylglyoxime.

Buffer. A buffer solution having a pH of 6.0 was prepared by adding 363 ml. of 0.5 *M* sodium hydroxide to 500 ml. of 0.4 *M* potassium biphthalate and diluting to 1 liter with water. The pH of the buffer is practically unchanged when diluted from 25 to 100 ml.

All other reagents used were of the highest purity obtainable.

Experimental

The usual procedure followed in this work was to transfer the desired quantity of cobalt to a 100-ml. volumetric flask, add 25 ml. of buffer and 5 ml. of the reagent, and dilute to the mark with water. After thorough mixing, color comparisons were made in Nessler tubes and transmittancy measurements made on another portion of the solution.

Using Nessler tubes, 1 part of cobalt in 20,000,000 parts of solution may be determined at cobalt concentrations of 0 to 0.08 mg. per 100 ml.; 1 in 10,000,000 at 0.08 to 0.2 mg.; 1 in 5,000,000 at 0.2 to 0.25 mg. These are also the approximate increments to be employed in making up a standard series. The spectrophotometer is slightly more sensitive; 1 part of cobalt in 50,000,000 of solution may be detected.

Transmittancy curves for solutions of the reagent and of the cobalt complex are given in Figure 1. The concentration of the reagent must be kept relatively low to prevent a decrease

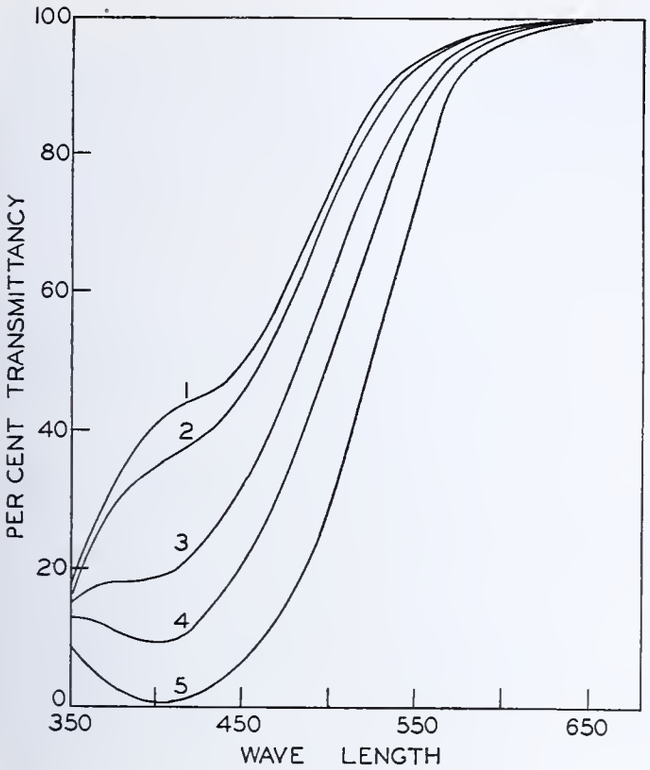

FIGURE 1. EFFECT OF COBALT CONCENTRATION

1. 25 p. p. m. of reagent, no Co
2. 0.1 p. p. m. of Co
3. 0.5 p. p. m. of Co
4. 1 p. p. m. of Co
5. 2.5 p. p. m. of Co

in the sensitivity for cobalt, because the reagent is highly colored and absorbs strongly in the same spectral region as the cobalt complex. The reaction rate, however, is prohibitively slow at very low reagent concentrations. The concentration of reagent recommended, 2.5 mg. per 100 ml., results in immediate color formation and also permits the determination of small amounts of cobalt.

The maximum amount of cobalt determinable is limited by the relatively small amount of reagent employed. No increase in intensity occurs if the quantity of cobalt present exceeds 0.26 mg. with 2.5 mg. of reagent. These quantities correspond to a molar ratio of reagent to cobalt of slightly greater than 3 to 1. This indicates that the compound formed is an inner complex of nitrosoresorcinol and the cobaltic ion, similar to that obtained with β-nitroso-α-naphthol.

The maximum difference between the transmittancy of solutions of the reagent and of the cobalt complex occurs at a wave band of 420 to 430 mμ at low cobalt concentrations. Most of the measurements were made in this region. A wave length of 450 mμ may be advantageously used for cobalt concentrations above 0.15 mg. per 100 ml. The validity of Beer's law at 430 and 450 mμ is shown in Figure 2.

EFFECT OF pH. The color of the reagent varies markedly with the pH of the solution. Below pH 2.5 it has a pale greenish-yellow color which increases in intensity and becomes orange as the pH increases from 2.5 to 5.6. From 5.6 to 6.5 little change in the intensity occurs, but it increases slightly at 7.0. A further increase in pH results in a slight decrease in intensity. The transmittancy curves of solutions of the reagent at various pH's are given in Figure 3. No break in the curve at a wave length of 440 mμ is observable at a pH of 4.0.

The pH also influences the intensity of the red color of the cobalt complex. No visible reaction occurs below a pH of 2.0. The intensity of the color increases from 2.0 to 5.6, is practically constant from 5.6 to 6.3, and decreases slightly with increasing pH above 6.5. A pH of 6.0 was employed in all experiments, although the method is applicable at a pH range of 5.6 to 6.5. Figure 4 shows the transmittancy curves of solutions of the cobalt complex at various pH's.

To afford increased buffer capacity, the concentration of the buffer may be increased without any effect on the intensity of the color. A biphthalate–sodium hydroxide buffer was employed in preference to a phosphate or citrate buffer because the presence of phosphate or citrate causes a decrease in the intensity of the color of the cobalt complex.

The rate of reaction is dependent on the pH of the solution, being slower at a pH of less than 5.6 than above this value. The reaction occurs practically instantaneously at a pH of 6.0 and above.

STABILITY. Solutions of the cobalt complex are stable for at least a month. The transmittancy, at 450 mμ, of a solution containing 0.15 mg. of cobalt was 0.128 when prepared and remained unchanged for a month. The transmittancy of solutions of the reagent changes slightly on long aging—for example, the transmittancy at 450 mμ increased from 0.488 to 0.510 after a month. The transmittancy increases slightly at wave lengths less than 500 mμ but decreases at longer wave lengths on aging. Visually, solutions of the reagent fade slightly on long aging in the buffer or aqueous medium.

INTERFERENCE. In these studies the usual procedure was followed but the ion in question was added prior to the addition of the reagent. Observations were made in the absence of cobalt and at a cobalt concentration of 1 p. p. m.

The presence of 5 grams of sodium nitrate, potassium chloride, ammonium nitrate, sodium chloride, or ammonium

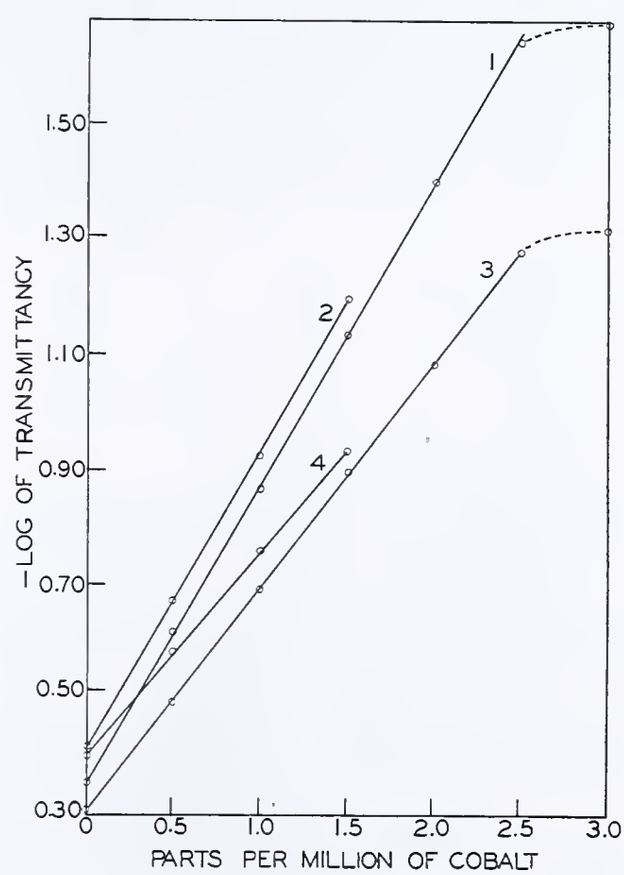

FIGURE 2. TEST OF BEER'S LAW WITH COBALT-NITROSORESORCINOL SOLUTION

1. 430 mμ
2. 430 mμ, 50 p. p. m. of Ni
3. 450 mμ
4. 450 mμ, 50 p. p. m. of Ni

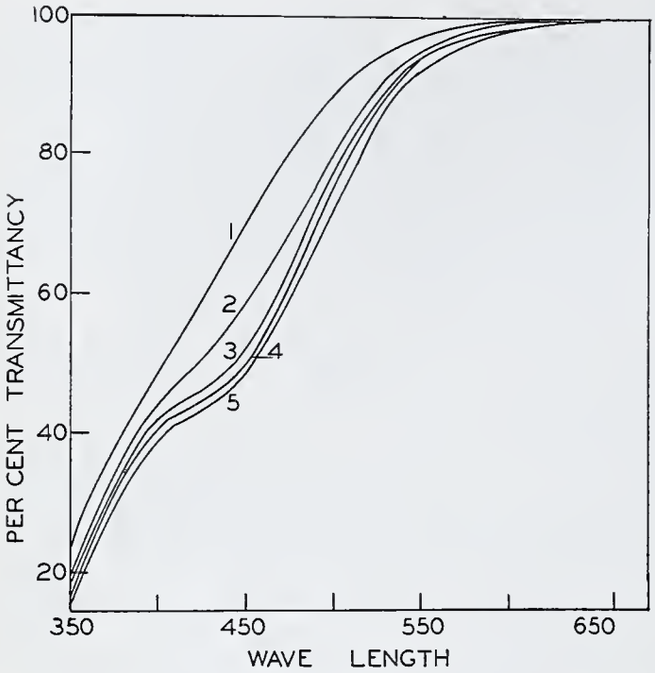

FIGURE 3. EFFECT OF pH ON NITROSORESORCINOL SOLUTIONS

1. pH = 4.0
2. pH = 4.6
3. pH = 5.2
4. pH = 5.6–6.3
5. pH = 7.0 (25 p. p. m. of reagent)

chloride had no effect on the transmittancy of the solutions at 430 mμ. Visually, the presence of more than 1 gram of ammonium chloride produced a green tint. All the other salts cause a similar off shade of color on standing. However, if the color comparisons are made within an hour the salts cause no interference. The concentration of various ions that may be present without interference is given in Table I.

TABLE I. CONCENTRATIONS NOT INTERFERING

Ions	Concentration *P. p. m.*
Ba^{++}, Ca^{++}, Mg^{++}, Pb^{++}	100
Mn^{++}, Th^{++++}, Yb^{+++}, Yt^{+++}	10
Cr^{+++}	5
Ag^{+}, Al^{+++}, Hg^{+}, Hg^{++}	1
Ti^{++++}, Zr^{++++}	0.5

The interference of the other common metals is considered in more detail in the following sections.

Copper. The cupric ion reacts with *o*-nitrosoresorcinol, giving a complex that is nearly as intense in color as that with cobalt. From the transmittancy curve for a solution of the copper complex (Figure 5) it is evident that no wave band may be employed that will eliminate the interference of the cupric ion. The technique used for determining cobalt in the presence of nickel, described below, is not applicable for copper. Apparently, the complex formed with the cupric ion is more stable than that with nickel. The quantity of copper present must not exceed 0.01 to 0.02 mg. in either the visual or spectrophotometric methods.

Zinc. The transmittancy curve for the zinc complex is given in Figure 5. The reagent is not sensitive for zinc, a concentration of 20 p. p. m. not interfering in the visual method. A zinc concentration of 1000 p. p. m. causes no interference, if the transmittancy measurements are made at a wave band of 420 mμ. The high zinc concentration does cause a decrease in the rate of formation of the cobalt complex. Thus, at a zinc concentration of 1000 p. p. m. it is necessary to allow the solutions to stand 3 to 4 hours before making the measurements if the cobalt concentration is 1 p. p. m. At lower cobalt or zinc concentrations a shorter period of time is required. No evidence of the precipitation of zinc hydroxide was observed, even at a zinc concentration of 5000 p. p. m.

Cadmium. A solution of the cadmium complex gives a transmittancy curve similar to that for zinc, as seen in Figure 5. Visually, a cadmium concentration of 100 p. p. m. causes no interference; 1000 p. p. m. of cadmium cause no interference if the transmittancy measurements are made at a wave band of 425 mμ. The measurements may be made immediately, since this high cadmium concentration does not decrease the reaction rate of cobalt with the reagent. The cadmium concentration must be limited to approximately 1000 p. p. m. to avoid possible precipitation of the hydroxide.

Palladium. The transmittancy curve for a solution of the palladous complex given in Figure 5 is similar to that for the cobalt complex. The intensity of the colored palladous complex at low concentrations is almost as great as that of the cobalt compound. The reagent is not recommended for palladium, however, because the complex is slow in forming and the colored solutions are not stable after the complex has formed. Palladium must be absent to avoid interference.

Iron. The reagent reacts with ferric ions, giving a green solution at a concentration as low as 0.1 p. p. m. The use of citrate or tartrate does not completely eliminate this interference. The intensity of the colored solution increases and the transmittancy decreases slowly on aging. The interference due to iron can be eliminated by precipitating the ferric iron with potassium fluoride and filtering. Unfortunately, some of the ferric salts, from different sources, contained an unidentified interfering substance. Solutions prepared from ferric nitrate nonahydrate or ferric ammonium sulfate dodecahydrate caused no interference, visually or spectrophotometrically. Those prepared from ferric chloride hexahydrate or iron wire did interfere, producing slightly darker colored solutions than obtained in the absence of iron. This interference could be eliminated by purifying the iron salts before use.

The reagent is not recommended for cobalt if iron is present, because it is impossible to know whether or not the interfering substance accompanies the iron. The results obtained, however, do show that a clean-cut separation of cobalt from iron is possible by precipitating the iron as the complex fluoride—for example, no cobalt is lost when the iron is precipitated from a solution containing 500 mg. of iron and 0.05 mg. of cobalt, a ratio of 10,000 to 1.

Determination of Cobalt in the Presence of Nickel

Nickel also forms a colored complex with *o*-nitrosoresorcinol under the conditions used for the determination of cobalt. The transmittancy curve for the nickel complex (Figure 5)

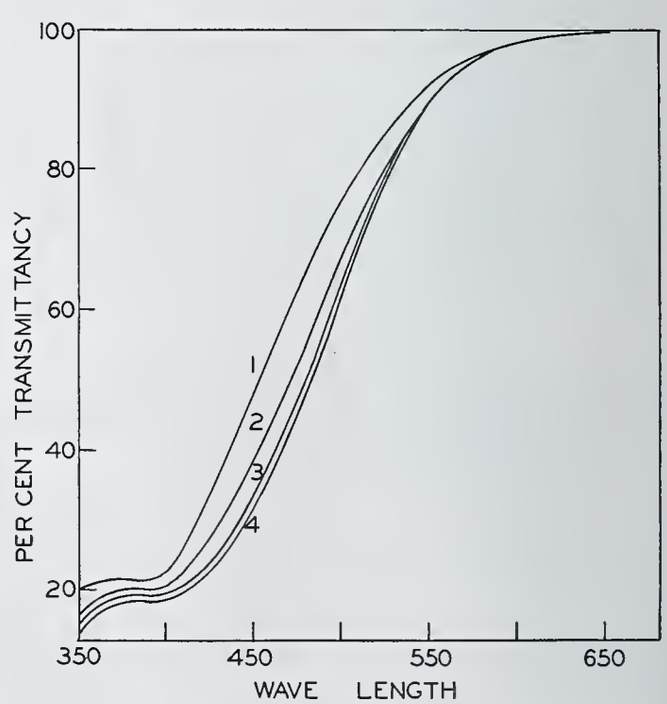

FIGURE 4. EFFECT OF pH ON COBALT-NITROSORESORCINOL SOLUTIONS

1. pH = 4.0
2. pH = 4.6
3. pH = 5.2 and 7.0
4. pH = 6.0 (0.5 p. p. m. of Co)

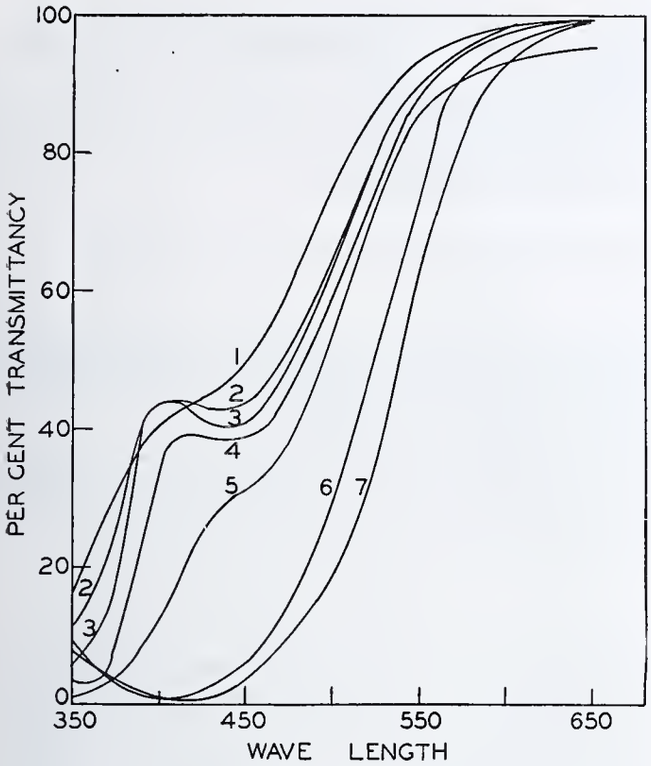

FIGURE 5. TRANSMITTANCY CURVES FOR SOLUTIONS OF METAL COMPLEXES OF NITROSORESORCINOL

1. 25 p. p. m. of reagent
2. 2000 p. p. m. of Cd
3. 2000 p. p. m. of Zn
4. 50 p. p. m. of Ni
5. 5 p. p. m. of Cu
6. 3 p. p. m. of Co
7. 10 p. p. m. of Pd

shows that at no wave band may the interference of nickel be effectively eliminated. The nickel concentration would have to be limited to 0.5 p. p. m., if this interference could not be eliminated by some method.

Using a constant cobalt concentration, the intensity of the color increases with increasing nickel concentration up to approximately 10 p. p. m. The intensity is less immediately after adding the reagent at higher nickel concentrations but increases on standing until the intensity is the same as at 10 p. p. m. This is understandable, if it is remembered that the total intensity of the color is the sum of that of the cobalt and nickel complexes and that the intensity of the cobalt complex is greater than that of the nickel at the same concentration. At the lower nickel concentrations, the cobalt complex forms immediately and any reagent in excess of that required for the cobalt forms the nickel complex simultaneously. At the higher nickel concentrations, however, the formation of the nickel complex is favored, but since the cobalt complex is more stable than the nickel complex a slow shift from the nickel to the cobalt complex occurs, resulting in the increase in the color intensity on standing.

The transmittancy of solutions of the nickel complex is practically independent of the nickel concentration, if the latter is above 10 p. p. m. This indicates that the concentration of nickel does not have to be controlled carefully, providing the concentration is above 10 p. p. m. The reaction rate, between the cobalt and reagent, however, decreases with increasing nickel concentration, thereby setting a practical limit to the amount of nickel that may be present.

An increase in the concentration of the reagent increases the reaction rate but results in a decrease in the sensitivity. This difficulty is avoided if the volume of the solution is kept below 50 ml. during the aging period and diluted to 100 ml. before making the measurements. The aging is carried out at approximately 100° C., because the reaction rate also increases with increasing temperature. Results of experimentation are given in the following procedure:

PROCEDURE FOR DETERMINING COBALT IN THE PRESENCE OF NICKEL. Transfer the test solution to a 100-ml. Erlenmeyer flask, and add 25 ml. of buffer and 5 ml. of reagent. Heat on a steam or water bath for a period of time depending upon the cobalt and nickel concentration present. Cool, transfer quantitatively to a 100-ml. volumetric flask, and dilute to the mark with water.

Using 5 mg. of nickel 2 hours of heating are required for a final cobalt concentration of 1 p. p. m.; 3 hours for 1.5 p. p. m. With 10 mg. of nickel 4 hours are required for 1 p. p. m. of cobalt; 6 hours for 1.5 p. p. m. For 15 mg. of nickel 6 hours are required for 1 p. p. m.; 10 hours for 1.5 p. p. m. of cobalt. A longer time is required if the volume of the solution is greater than 40 ml. during the heating period. It is impractical for the nickel concentration to exceed 150 p. p. m. and the cobalt concentration 1.5 p. p. m.

The use of a spectrophotometer is recommended. With this instrument it is possible to detect 0.002 mg. of cobalt in the presence of 10 mg. of nickel—i. e., 0.02 per cent of cobalt.

Some transmittancy values for mixtures of cobalt and nickel are given in Table II.

Additional results are shown in Figure 2 for various cobalt concentrations in the presence of 5 mg. of nickel. Beer's law is valid, although the lines have origins different from those obtained in the absence of nickel.

The visual method is applicable if appropriate amounts of nickel are present in the standards and the standards are heated for the proper periods of time. Visually, the method is slightly less sensitive for cobalt in the presence of nickel, owing to the increased intensity of the colored solutions.

The effect of potassium fluoride on the method was studied briefly in connection with the possible determination of cobalt in solutions also containing nickel and iron. The presence of potassium fluoride decreases the color intensity of the nickel complex, especially on heating. The aging can be carried out at room temperature, however, since the presence of potassium fluoride increases the reaction rate of the cobalt and reagent. The visual method is not applicable to mixtures of cobalt and nickel if potassium fluoride is present, but spectrophotometric measurements made at 450 mμ are satisfactory.

TABLE II. TRANSMITTANCY VALUES

Nickel Added	Cobalt Added	Transmittancy at 430 mμ
Mg.	*Mg.*	
0	0.05	0.246
0	0.10	0.130
0	0.15	0.071
1	0	0.403
5	0	0.401
10	0	0.402
1	0.05	0.218
5	0.05	0.213
10	0.05	0.213
1	0.10	0.121
5	0.10	0.120
10	0.10	0.122
1	0.15	0.063
5	0.15	0.068

Summary

A study of the reaction between cobalt and *o*-nitrosoresorcinol has shown that the reagent may be used for the colorimetric determination of cobalt. Spectrophotometric data for solutions of the reagent, for the cobalt complex, and for other metal complexes are presented. Procedures are given for the determination of cobalt alone and for cobalt in mixtures of nickel and cobalt.

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Polarographic Determination of Formaldehyde in Biological Material

Application to the Determination of Serine

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FORMALDEHYDE has been determined quantitatively by the polarograph according to Jahoda (*3*), Winkel and Proske (*5*), and Barnes and Speicher (*1*). Recently Boyd and Logan (*2*) have developed a colorimetric method for the determination of formaldehyde as a step in the procedure for estimating serine. Their method consists essentially of quantitatively separating by distillation the formaldehyde resulting from the action of periodate on protein hydrolyzates containing serine, and condensing this formaldehyde with chromotropic acid (Eegrewe's reagent) to form a colored compound.

TABLE I. DETERMINATION OF FORMALDEHYDE BY POLAROGRAPHIC AND COLORIMETRIC METHODS

Sample	Polarographic Method: In 3 ml. of distillate	Polarographic Method: Per ml.	Colorimetric Method: Per ml.
		Micrograms	
1 mg. of serine	8	2.7	2.7
1 mg. of serine	8.7	2.9	2.8
5 mg. of serine	41	13.7	14.1
5 mg. of serine + 100 mg. of threonine	42	14	14.1
5 mg. of serine + 100 mg. of threonine	42	14	14.2
50 mg. of serine	382	127	128.8
50 mg. of serine	385	128	131.6
Gelatin hydrolyzate	155	52	49.5
Gelatin hydrolyzate	155	52	48.7
Salmin hydrolyzate	20.5	6.8	6.5
Salmin hydrolyzate	21	7	6.7
Salmin hydrolyzate	22	7.3	6.5
Casein hydrolyzate	21	7.0	7.0
Casein hydrolyzate	22	7.3	7.0
Horse hemoglobin (crystallized) hydrolyzate	19	6.3	6.1
Horse hemoglobin (crystallized) hydrolyzate	19.5	6.5	6.4
Dog hemoglobin (crystallized) hydrolyzate	19	6.3	6.1

In order to ascertain whether the polarograph can be used to determine the formaldehyde found in the distillate in the above procedure, the authors compared polarographic analyses against those run colorimetrically. The polarographic procedure proved suitable for this type of determination, since no substances which interfered with the production of smooth current-voltage curves came over in the distillation.

Procedure

The formaldehyde is separated from the protein hydrolyzate and the periodate reaction mixture by distillation, as described by Boyd and Logan (*2*). Three milliliters of the distillate are mixed with 1 ml. of a solution of electrolytes (0.4 *N* potassium chloride, 0.2 *N* potassium hydroxide), and nitrogen is bubbled through the mixture for 3 minutes. This does not result in loss of formaldehyde, since repeated runs on the same solution showed that gassing for as long as 15 minutes did not change the height of the step. The polarogram of the solution is then taken from −1.4 to −1.8 volts with the Leeds & Northrup Electro-chemograph; the half-wave of the formaldehyde step occurs at about −1.63 volts (normal calomel electrode). Accurate temperature control is important; a constant-temperature water bath thermostatically controlled to 0.1° C. was used.

The authors' results confirm Barnes and Speicher's findings (*1*) that the wave height changes about 6.5 per cent for each 1° C. change in the temperature of the solution. The height of the step is measured in the usual way and compared with a calibration curve made with known quantities of formaldehyde at the same temperature and drop rate, and with the same capillary.

Discussion

The results obtained by the polarographic method agree with those obtained by the colorimetric method of Boyd and Logan (*2*) as shown in Table I. Quantities of formaldehyde as low as 3 micrograms per ml. of distillate can be determined by the polarographic procedure.

According to Barnes and Speicher, when potassium hydroxide is the only supporting electrolyte, the full height of the formaldehyde step is not given in concentrations of potassium hydroxide below 0.5 per cent, and so those workers used 1.25 per cent solutions in their determinations. In the authors' experience, satisfactory curves with the full height of the formaldehyde wave are obtained with 0.05 *N* potassium hydroxide in 0.1 *N* potassium chloride, so that it is not necessary to employ stronger alkali, thereby decreasing the possibility of condensation of the formaldehyde.

Acetaldehyde (which arises from the action of periodate on threonine, *4*) does not interfere in the analysis unless it is present in extremely large amounts, since it is not reduced until a potential of −1.8 volts is reached (Figure 1). This method cannot be used for the determination of small amounts of acetaldehyde unless the potassium ion in the supporting electrolyte is replaced by lithium, because the potassium wave interferes with that of acetaldehyde. However, good results can be obtained with acetaldehyde in lithium hydroxide. Other aldehydes, higher in the aliphatic series, are reduced at still higher voltages.

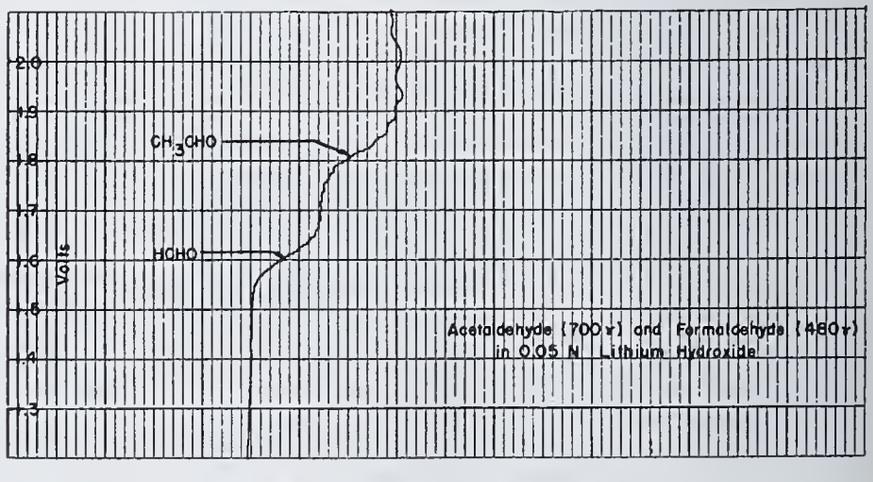

FIGURE 1. POLAROGRAM OBTAINED WITH LEEDS & NORTHRUP ELECTRO-CHEMOGRAPH

This method need not be limited to the determination of formaldehyde liberated by the action of periodate on serine. It can probably be applied to the determination of formaldehyde in other types of samples; as long as the compound can be isolated by distillation, reasonably free from interfering substances, it can be estimated polarographically.

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Determination of Small Amounts of Molybdenum in Tungsten and Molybdenum Ores

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A rapid method has been developed for the determination of small amounts of molybdenum in tungsten and molybdenum ores. After removing iron and other major constituents the molybdenum thiocyanate color is developed in water-acetone solutions, using ammonium citrate to eliminate the interference of tungsten. Comparison is made by titrating a blank with a standard molybdenum solution. Aliquots are adjusted to deal with amounts of molybdenum ranging from 0.01 to 1.30 mg.

MOST of the calcium tungstate ores from contact-metamorphic deposits in the western states contain at least a trace of molybdenum, and in many such ores as much as 0.5 per cent of molybdenum may be present. As concentrates from such ores are subject to a penalty, the determination of molybdenum is important.

In its search for new supplies of the different strategic metals the U. S. Geological Survey has examined various samples of possible molybdenum content that were on the border line between a little and no molybdenum. For such work a quick but accurate method was needed. One method developed for this purpose is based on fluorescence (*1*); the other depends on chemical analysis and colorimetric estimation of the molybdenum.

The amber color developed by molybdenum, thiocyanate, and a reducing agent has long been used as a sensitive test for molybdenum, the colored complex usually being concentrated by extraction with organic solvents such as ether, butyl acetate, or cyclohexanol, before colorimetric estimation. Ether, the most common solvent employed, has several objections, as noted by Sandell (*5*). The authors have found that even a few milligrams of tungsten or vanadium cause high results with the ether extraction alone, unless tartrate or citrate is present. Hurd and Allen (*3*) studied the conditions for maximum stability of color, using various solvents, and stated that "butyl acetate, although an excellent solvent for the thiocyanate, should be used with care. The color produced by a given concentration of molybdenum appears to depend to no slight extent upon the manner in which the solvent has been saturated with the reagents." They reported that cyclohexanol is difficult to handle, separates slowly from aqueous solution, and may develop a turbidity.

The writers dispense with extractions altogether, but employ an acetone-water solution, as acetone appears to have a stabilizing action and prevents fluctuations of the color with time. The interference of tungsten is eliminated by the use of ammonium citrate.

The essential features of the procedure are that the molybdenum thiocyanate color is developed in a water-acetone solution and the comparison is made by titrating a blank containing the necessary reagents with a standard molybdenum solution.

Reagents

STANNOUS CHLORIDE (10 PER CENT). Dissolve 20 grams of stannous chloride dihydrate in 100 ml. of concentrated hydrochloric acid and 100 ml. of water.

AMMONIUM THIOCYANATE (10 PER CENT). Dissolve 20 grams in 200 ml. of water.

AMMONIUM CITRATE, reagent grade.

ACETONE, reagent grade.

STANDARD MOLYBDENUM SOLUTION. 1 ml. = 0.1 mg. of molybdenum trioxide. Dissolve 0.1 gram of pure molybdenum trioxide in a little sodium hydroxide, neutralize to litmus with hydrochloric acid, and make up to 1 liter in a volumetric flask. As the standard degenerates with time, it is necessary to make up a fresh solution every month.

Solution of Sample

With substances like scheelite that contain up to 40 per cent of tungstic oxide and possibly as much as 1 per cent of molybdenum oxide, fuse in platinum 0.5 gram of the sample with 2.5 grams of sodium carbonate and about 0.05 gram of sodium nitrate (*2*). Begin the heating at a low temperature and gradually increase the heat until a homogeneous melt is obtained. Extract the cooled melt with water containing a drop of alcohol to reduce manganate, add a little paper pulp, filter, and wash with warm 1 per cent sodium carbonate solution. Make just acid with hydrochloric acid. If any chromate is present, reduce it with a little sulfurous acid. Evaporate to about 25 ml. on the steam bath to remove carbon dioxide. At this point silica may separate out. Without removing silica neutralize with 10 per cent sodium hydroxide and add 10 ml. in excess, add a little paper pulp, and digest on the bath about 10 minutes. Filter hot and wash with dilute sodium hydroxide. Add phenolphthalein to the filtrate, make it barely acid with hydrochloric acid, and transfer it to a 100-ml. volumetric flask. The solution is now ready for the estimation of molybdenum.

With molybdenite ores, wulfenite ores, and ores high in silica, in which tungsten is low, an acid attack of the sample is preferable. The usual practice (*6*) involves treatment with nitric and sulfuric acids and eventual extraction of the molybdenum in a sulfuric acid solution. On a 0.5-gram sample, 2 ml. of sulfuric acid are sufficient.

Colorimetric Estimation of Molybdenum

Take a 15-ml. aliquot of the neutral molybdenum solution in a 100-ml. beaker; if a larger or smaller aliquot is taken, adjust volume to 15 ml. Add to the solution at room temperature 1.5

grams of ammonium citrate and stir until it is dissolved. Now add 5 ml. of 10 per cent ammonium thiocyanate and 25 ml. of acetone, cool, add dropwise by pipet exactly 7 ml. of stannous chloride solution, and stir. Accurate measurement is important, as the acidity of the solution is controlled by the quantity of stannous chloride added. This develops the amber color of the molybdenum thiocyanate. To a blank in another 100-ml. beaker add 15 ml. of water, 1.5 grams of ammonium citrate, 5 ml. of ammonium thiocyanate, and 25 ml. of acetone. Add by pipet exactly 7 ml. of stannous chloride solution. Titrate this blank to a color match by adding standard molybdenum solution from a buret.

Occasionally the unknown will precipitate silica on the addition of stannous chloride, especially if the addition is too rapid. If this occurs, filter off the silica at this point before matching the color.

Results with Known Mixtures

In the experimental work accurately measured quantities of solutions of known titer, containing the sodium salts of the elements in question, were transferred to 100-ml. beakers. The solutions were adjusted to 15 ml. and made just acid before the addition of the reagents used for the development of the color. Estimations were made by titrating blanks with standard molybdenum solution.

Table I lists the results obtained in the presence of tungsten. The color is stable in the presence of tungsten for at least 3 hours.

Table I. Estimation of Molybdenum in the Presence of Tungsten

No.	Taken WO_3	Taken MoO_3	MoO_3 Found
	Gram	*Mg.*	*Mg.*
1	None	0.06	0.06
2	None	0.20	0.19
3	None	0.50	0.51
4	None	0.80	0.82
5	0.20	0.03	0.03
6	0.20	0.06	0.06
7	0.20	0.10	0.11
8	0.20	0.40	0.39
9	0.20	0.70	0.72
10	0.20	1.00	1.02
11	0.20	1.50	1.43
12	0.40	0.03	0.03
13	0.40	0.40	0.39
14	0.40	0.70	0.69

Interference

Iron causes a serious positive error and must be removed completely. The error is not so much a function of the quantity of iron present as of the time elapsed before the sample is matched. Table II illustrates this interference of iron when the solution is matched immediately. Table III shows the interference as a function of time.

Iron as well as many other interfering bases is, however, removed at the start by the carbonate fusion. Small or moderate amounts of iron are further removed with sodium hydroxide as described in the procedure. Excess ammonium hydroxide may be substituted for sodium hydroxide but the digestion is best conducted in a stoppered Erlenmeyer flask. In four experiments, 0.05, 0.30, 1.00, and 50.0 mg. of iron in the presence of 0.3 gram of tungstic oxide were completely removed by this method. Phosphate causes no interference when tungsten is absent. With tungsten present the color fades and turns blue-green with time. If matched within 10 minutes, 20 mg. of phosphorus pentoxide in the final aliquot with 0.3 gram of tungstic oxide show negligible interference. With 0.1 gram of tungstic oxide, 80 mg. of phosphorus pentoxide cause little trouble during this 10-minute interval. The interference of phosphate may be minimized by using more ammonium citrate.

Large amounts of vanadium interfere because of the blue-green color of vanadium in reduced solutions. Amounts of vanadium up to 2 mg. of vanadium trioxide in the final aliquot, however, do not appreciably affect the molybdenum color. Arsenic, antimony, sodium, and ammonium salts, calcium, aluminum, and silica do not interfere. Nitrates tend to cause a fading of color with time. Preferably the final solution should contain less than 0.3 gram of nitrate.

Table II. Interference of Iron

(Sample matched immediately)

No.	Taken WO_3	Taken Fe as $FeCl_3$	Taken MoO_3	MoO_3 Found	Error
	Gram	*Gram*	*Mg.*	*Mg.*	*Mg.*
1	0.3	0.02	0.03	0.08	+0.05
2	0.3	0.07	0.03	0.09	+0.06
3	0.3	0.02	0.10	0.19	+0.09
4	0.3	0.07	0.10	0.20	+0.10
5	0.3	0.02	0.30	0.52	+0.22
6	0.3	0.03	0.40	0.65	+0.25
7	0.3	0.05	0.40	0.65	+0.25
8	0.1	0.05	0.40	0.63	+0.23
9	0.1	0.10	0.40	0.64	+0.24
10	0.3	0.0001	0.50	0.55	+0.05
11	0.3	0.0002	0.50	0.60	+0.10
12	0.3	0.0010	0.50	0.75	+0.25

Table III. Interference of Iron with Time

No.	Taken WO_3	Taken Fe	Taken MoO_3	MoO_3 Found: Matched immediately	MoO_3 Found: Matched after 15 minutes
	Gram	*Gram*	*Mg.*	*Mg.*	*Mg.*
1	0.3	0.07	0.30	0.55	0.65
2	0.3	0.07	0.40	0.68	0.89
3	0.3	0.07	0.50	0.77	1.10

Determination of Molybdenum in Synthetic Scheelite

K. J. Murata of this laboratory prepared pure synthetic members of the scheelite-powellite mineral series by the method of Michel (*4*), and these were analyzed for molybdenum. The ground samples were attacked by 10 ml. of hydrochloric acid and 2 drops of nitric acid, evaporated to dryness, and taken up with 1.5 grams of ammonium citrate and 4 drops of ammonium hydroxide. The color was then developed and compared.

The results, as indicated in Table IV, showed good agreement with the synthetics.

Table IV. Determination of Molybdenum in Synthetic Scheelite

Preparation No.	MoO_3 in Synthetic Scheelite	MoO_3 Found
	%	%
4	0.07	0.07
26	0.22	0.23
28	0.25	0.24
6	0.29	0.31
27	0.36	0.34
29	0.36	0.34
7	0.51	0.50
31	0.72	0.68
5	0.79	0.74

Acknowledgment

The authors wish to thank K. J. Murata for kindly supplying samples of synthetic scheelite of known molybdenum content.

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Quantitative Estimation of Acetyl in Carbohydrate Acetates

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METHODS for the quantitative determination of carbohydrate acetyl have been developed principally along two separate lines: (1) for the simple sugar acetates which require the use of mild conditions to prevent errors which might arise from the destruction of the labile carbohydrate molecules; (2) for cellulose acetates which, because of the heterogeneous reaction involved, require somewhat more drastic conditions or considerably extended reaction time. Separate, essentially noninterchangeable methods were, therefore, evolved for the two classes of carbohydrate derivatives. Adaptations of the aforementioned methods have been applied to starch acetates, but none appears to have received general acceptance.

This paper describes a quantitative method of acetyl analysis which is applicable not only to the polysaccharide acetates of starch and cellulose but, on slight modification, to the simple sugar acetates as well. The method employs the sodium methoxide catalyst of Zemplén (*7*) to transesterify carbohydrate acetyl in methanol with the production of methyl acetate which is then quantitatively removed and determined by a modification of the well-known Freudenberg (*2*) procedure. Starch and cellulose do not yield volatile acidic decomposition products on contact with alkaline catalyst during the separation of the methyl acetate by distillation. The lower sugars, being labile, are decomposed on boiling in the presence of the catalyst. Therefore, in these cases, the transesterification is accomplished at room temperature, and the catalyst is neutralized by the addition of a methanol solution of sulfuric acid before proceeding with the distillation.

The transesterification is rapid, requiring only 15 minutes at room temperature, for acetates of the simple carbohydrates. Even in the case of polysaccharide acetates, which remain insoluble, the reaction is completed in about 25 minutes. In certain instances with dense or somewhat horny acetates the reaction proceeds slowly. In these cases it is expedient first to swell the acetate by heating in methanol for 0.5 to 1 hour before proceeding with the analysis.

The apparatus employed for the analysis is a modification of the Freudenberg (*2*) apparatus as shown in Figure 1.

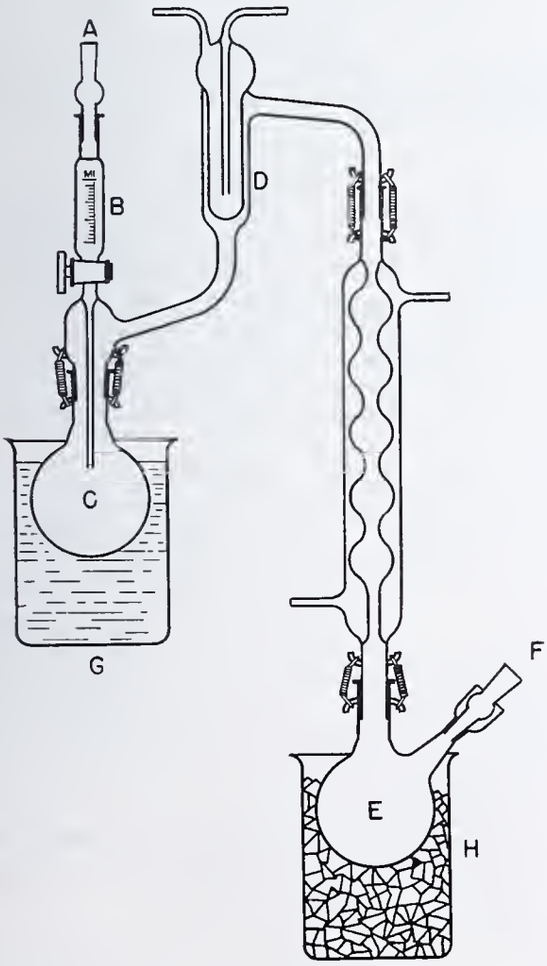

FIGURE 1. APPARATUS

Analytical Procedure

REAGENTS. Anhydrous methanol was prepared by drying methanol with magnesium methoxide (*4*) and distilling through a fractionating column.

Sodium methoxide was prepared by dissolving clean sodium in anhydrous methanol. The sodium was cut under xylene to expose fresh surfaces and weighed. It was then rapidly passed through three separate portions of anhydrous methanol contained in beakers and finally dissolved in anhydrous methanol in a volumetric flask and made up to volume. Approximately 0.2 *N* solutions were employed.

An approximately 0.2 *N* solution of sulfuric acid in methanol was prepared by adding a calculated amount of concentrated sulfuric acid to anhydrous methanol and allowing the mixture to stand several days to permit formation of monomethyl sulfate. The solution was then standardized and was found to maintain its normality for a considerable time.

PROCEDURE FOR POLYSACCHARIDE ACETATES. A 0.2- to 0.3-gram sample of finely divided polysaccharide acetate, previously dried in vacuo over phosphorus pentoxide, is placed in 20 ml. of anhydrous methanol in the reaction flask (*C*, Figure 1), containing a boiling tube to prevent bumping. Approximately 5 to 10 ml. of 0.2 *N* sodium methoxide (3 to 8 milliequivalents per gram of sample) in anhydrous methanol are added through the dropping funnel, *B*, which is protected by a small drying tube, *A*. The temperature of the water bath, *G*, is raised until slow distillation into the receiving flask, *E*, occurs, the rate of distillation being approximately 35 drops per minute. During the distillation, the receiving flask is cooled by immersion in crushed ice. The receiving flask may be protected by a small tube, *F*, filled with soda lime. When the liquid in the distillation flask has been concentrated to a small volume (approximately 5 ml.) a fresh 20-ml. portion of anhydrous methanol is admitted through the dropping funnel and the solution is again distilled to a small volume. Two 10-ml. portions of anhydrous methanol are then added successively and the solution is concentrated to a small volume after the addition of each portion. During the concentration the water level in the water bath is maintained just below the level of liquid in the distillation flask in order to prevent baking of material on the flask walls.

After the final concentration, the distillation flask is removed or the stopcock on dropping tube *B* is opened, and 25 ml. of 0.2 *N* sodium hydroxide are pipetted into the receiving flask by way of the side neck and the neck closed by a glass stopper. The bath, *H*, of crushed ice is removed and a hot water bath substituted. The contents of the receiving flask are refluxed 15 minutes, the flask is cooled, 75 ml. of boiled distilled water are added, and the excess sodium hydroxide is titrated with 0.1 *N* hydrochloric acid to a phenolphthalein end point. The base consumed in the saponification, minus that consumed in a separate blank determination, is equivalent to the acetyl content of the carbohydrate acetate. Blank determinations are made by refluxing 50 ml. of

methanol 15 minutes with 25 ml. of 0.2 *N* sodium hydroxide and titrating as described above.

It is not necessary that the catalyst remain in the reaction mixture throughout the distillation. After the first concentration of the liquid in the distillation flask the catalyst may be neutralized or the solution slightly acidified by addition of sulfuric acid dissolved in methanol, and the remainder of the distillation carried out as described. This procedure causes no change in the observed acetyl value. Samples not in a state of fine subdivision are refluxed with methanol for 0.5 to 1 hour before introducing the catalyst and proceeding with the analysis. For the reflux, water is circulated through condenser *D* but at all other times this condenser remains empty.

Procedure for Simple Sugar Acetates. A 0.20- to 0.25-gram sample of sugar acetate is added to the reaction flask and dissolved in 20 ml. of anhydrous methanol by gentle heating. When complete solution has occurred the mixture is cooled to room temperature, and 5 to 10 ml. of 0.2 *N* sodium methoxide in anhydrous methanol are added and well mixed with the solution. After 15 minutes, slightly more of a solution of sulfuric acid in methanol is added than is needed to neutralize the catalyst. The methyl acetate is then quantitatively distilled and determined as described in the procedure for polysaccharide acetates. The sample may be left in contact with the catalyst for periods longer than 15 minutes without affecting the results. The equilibrium effected by the catalyst apparently produces methyl acetate to the almost complete exclusion of carbohydrate acetate.

Discussion

Since the presence of sodium hydroxide in the solution would tend to prevent quantitative transesterification and distillation of the methyl acetate, it is important that water be carefully excluded from the reaction flask and that dry samples and anhydrous reagents be employed.

The amount of catalyst employed need not be precisely controlled. A several-fold variation in the ratio of catalyst to sample may be used without detriment to the analytical results. In general, 3 to 8 milliequivalents of catalyst per gram of sample were employed. Barium methoxide gives good results with the simple sugar acetates, but is not so effective with the polysaccharide acetates as is sodium methoxide.

Methanol is used in preference to ethanol because of the more favorable distillation of methyl acetate from a solution in methanol as compared to ethyl acetate from ethanol.

Results are reproducible to about ±0.1 per cent acetyl—for example, the value reported in Table I for cornstarch acetate I represents the mean value of 18 determinations all values of which were within ±0.2 per cent of the mean value and 15 results were within ±0.1 per cent. The accuracy of the results is good in the case of the lower sugar acetates. Since no method exists for definitely establishing the acetyl content of starch and cellulose acetates, the accuracy of the results in the case of these polysaccharides cannot be precisely determined. For this reason it was of interest to compare the values obtained for the polysaccharide acetates with values obtained by the use of a previously widely used procedure. The methods in the literature, applying principally to the determination of acetyl or combined acetic acid in cellulose acetates, have been comprehensively reviewed and criticized by several workers (*3*, *5*, *6*). The method recommended (*3*, *6*) as giving the most reliable results is a modification of the method of Eberstadt (*1*), which consists in saponification of the acetate in standard alkali and subsequent titration of the excess alkali with acid.

Values obtained from quadruplicate determinations by this method were compared with values resulting from the sodium methoxide procedure. In general, the results obtained by the two methods were in good agreement (Table I). In several cases, however, the modified Eberstadt method gave acetyl percentages 0.2 to 0.4 higher than the values obtained by the sodium methoxide procedure. In these instances, a blank run on the unacetylated starches showed that the samples possessed an acidity equivalent to 0.2 to 0.4 per cent acetyl. Acidic groups may result from the presence of fats, phosphoric acid residues, or carboxyl groups produced by partial oxidation of the carbohydrate. Correction for these acidities brought the results into agreement with those of the sodium methoxide procedure. Acetyl values which were thus corrected are indicated in Table I. Since cornstarch acetate III was a commercial product and none of the unacetylated starch was available, correction of the acetyl value for acidic contaminants could not readily be made. This acetate was impure, as indicated by its brown color. In contrast to the Eberstadt method, the sodium methoxide procedure is not affected by the presence of nonvolatile acidic contaminants such as fats and phosphoric acid residues or by the existence of free carboxyl groups resulting from oxidation of the polysaccharide.

Table I. Analysis of Carbohydrate Acetates[a]

Substance	Per Cent Acetyl: By sodium methoxide	By Eberstadt method	Theoretical
Cornstarch acetate I	44.36	44.48[b]	44.78[c]
Cornstarch acetate II	44.17	44.25[d]	...
Cornstarch acetate III[e]	36.94	37.64	...
White potato starch acetate	44.15	44.20[d]	...
Waxy cornstarch acetate	43.90	44.04[b]	...
Wheat starch acetate	44.31	44.10[d]	...
Cellulose acetate I[e]	40.51	40.21	...
Cellulose acetate II[e]	44.20	43.97	...
Glucose penta-acetate	55.23	...	55.13
Mannose penta-acetate	55.19	...	55.13
Maltose octa-acetate	50.79	...	50.74
Gentiobiose octa-acetate	50.77	...	50.74

[a] All values are average of at least triplicate determinations.
[b] No acidity found present in unacetylated starch.
[c] Calculated for $[C_6H_7O_4(CH_3CO)_3]$.
[d] Corrected for acidity in unacetylated starch.
[e] Commercial preparations.

Attempts to adapt the Freudenberg procedure (*2*) to polysaccharide acetates were not entirely successful. The slowness of the *p*-toluenesulfonic acid to cause transesterification required long reflux periods which resulted in abnormally large blank values. However, when the proper blanks were subtracted the acetyl values were in rough agreement with those indicated in Table I.

Summary

A method is reported for the quantitative estimation of acetyl in either polysaccharide acetates or simple sugar acetates. The acetates are transesterified by sodium methoxide catalyst in anhydrous methanol to yield methyl acetate, which is quantitatively distilled, and saponified in standard alkali. The method is rapid, gives reproducible results, and possesses the advantage of yielding acetyl values which are not affected by the presence of nonvolatile acidic groups in the sample.

Acknowledgment

The authors wish to thank P. A. Haeder and N. H. Koenig for performing some of the analytical determinations.

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Estimation of Volatile Acyl Groups in Cellulose Esters

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GENUNG and Mallatt (*4*) not only reviewed the literature of this important estimation but also submitted the principal method, that of Ost, to a critical experimental study. In this method, the cellulose ester is completely dissolved in cold, aqueous sulfuric acid (1 to 1), the solution containing the liberated organic acid, or acids, is distilled with steam, and the acidity of the distillate is found by titration.

During the past five years (*1*), several operators in this laboratory have obtained reliable and concordant analyses for the volatile acyl content of various cellulose esters by the following procedure, which is based on the work of Zemplén (*9*), Perkin (*7*), Freudenberg and Harder (*2*), and particularly Phillips (*8*). The acyl groups are recovered as methyl esters by a transesterification in methanol, with sodium methylate followed by excess *p*-toluenesulfonic acid as catalysts. Since the lower methyl esters are more volatile in methanol than the free acids are in steam, a short distillation, instead of the rather tedious one characteristic of the Ost process, transfers the esters to the distillate. This distillate is trapped in an excess of standard aqueous alkali. Saponification, removal of excess methanol by distillation, and titration of the residual aqueous solution complete the estimation.

Experimental

MATERIALS. Pure, dry methanol, free from aldehydes and acids, was prepared by heating the commercial C. P. grade under reflux for 6 hours with a few grams per liter of clean magnesium ribbon. Pellets of sodium hydroxide, mixed with clean aluminum turnings, could also be used. The methanol recovered by distillation in all-glass equipment was stored in glass-stoppered bottles.

If the purity of the commercial *p*-toluenesulfonic acid hydrate was doubted, the aqueous solution was filtered through absorbent charcoal and steam-distilled until the distillate was no longer acid (*8*). When crystallization from the highly concentrated still residue was complete, the crystals were recovered and were dried in vacuo over concentrated sulfuric acid. The stock solution contained equal weights of water and of the sulfonic acid hydrate.

Aqueous caustic soda, 0.2 *N*, and sulfuric acid, 0.1 *N*, were standardized against a National Bureau of Standards sample of acid potassium phthalate. The sodium methylate solution was prepared by dissolving the clean metal in the purified methanol and the normality was adjusted to 0.1 *N* after titrating an aliquot with standard acid.

APPARATUS. The apparatus was a modification of that used by Phillips (*8*) and was constructed entirely of Pyrex. Soft glass and rubber were avoided lest alkali from the former, or traces of hydrogen sulfide from the latter, reduce the accuracy of the estimation.

The 250-cc. round-bottomed flask, *A*, was sealed, as shown in Figure 1, to a 35-cc. dropping funnel and a No. 11 ground-glass joint. The joint fitted the similar one on the bottom of the 40-cm. (16-inch), vertical Liebig condenser, *B*, the top of which was sealed to the top of the similar condenser, *E*, through a horizontal tube about 20 cm. in length. An inverted 25-cc. Erlenmeyer flask at the bottom of *E* acted as a trap in case of a suck-back during distillation, and the exit, *D*, was sealed through a No. 20 ground joint. This joint fitted the one on the neck of the ellipsoidal receiver, *C*, which was blown from a 500-cc. round-bottomed flask. Powdered glass was fused inside the lower part of this receiver to promote uniform boiling during distillations. When the receiver was attached to the exit tube, *D*, in the position shown, the end of the tube remained above the level of the liquid in *C*. Rotation of the latter through 180° about the glass joint caused the end of the exit tube to become immersed.

The equipment was not particularly fragile when kept clamped to its vertical iron stand and accidental breakages were very few.

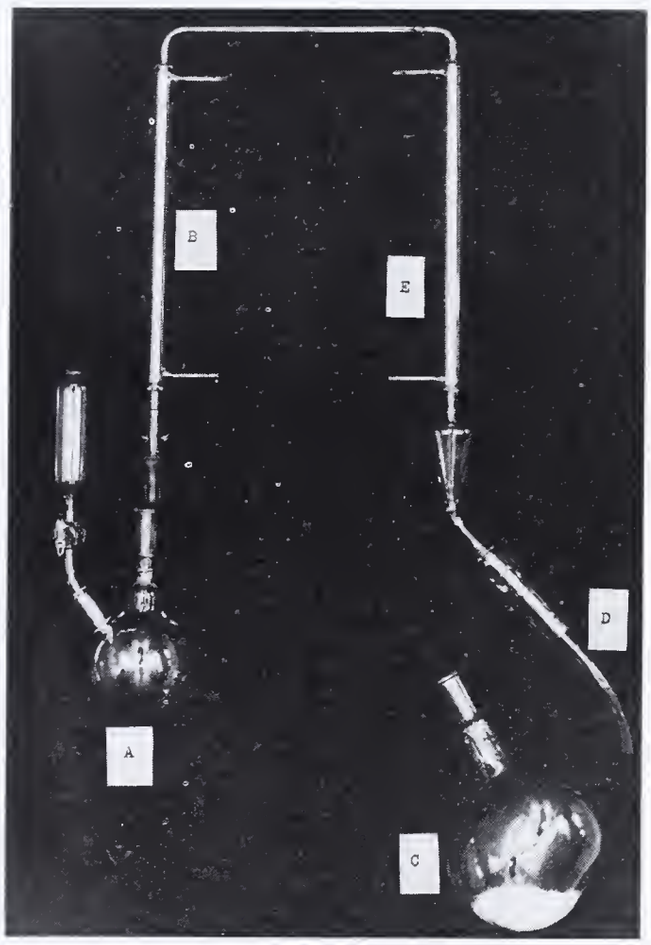

FIGURE 1. APPARATUS

ANALYSIS. After the cellulose ester, which had to be finely divided, had been thoroughly dried at 60° C. over phosphorus pentoxide in vacuo, a weighed sample, 0.3 to 0.4 gram, was put into flask *A*, together with 50 cc. of methanol, 10 cc. of the sodium methylate solution, and two or three glass beads. The flask was held in position on condenser *B* by means of rubber bands and the contents were kept in gentle ebullition for 3 hours by heat from a small flame. Cooling water was meanwhile circulated in condenser *B*. A cellulose acetate with which this preliminary transesterification was omitted analyzed 24.6 instead of 39.2 per cent acetyl.

After 3 hours, flask *C*, containing an accurately measured volume, 25 cc., of the caustic soda solution, was very loosely attached to *D*, so that the exit tube dipped below the surface of the liquid. The lower part of the flask was immersed in an ice bath and cold water was circulated in condenser *E*. Condenser *B*

[1] Present address, Du Pont Rayon Company, Buffalo, N. Y.
[2] Present address, Tennessee Eastman Company, Kingsport, Tenn.

was emptied. A large excess, 3 cc., of the *p*-toluenesulfonic acid solution was added through the dropping funnel attached to *A* and the mixture of methanol and methyl ester was slowly distilled into *C*. When the still residue had been reduced to about 15 cc., an additional 80 cc. of methanol was added dropwise from the funnel at such a rate that the volume in the still remained at 20 to 25 cc. The stopcock on the dropping funnel was then left open and receiver *C* was firmly attached by rubber bands to *D*, so that the exit tube remained above the surface of the liquid. After the ice bath was replaced by a small flame under an asbestos sheet, the methyl esters in *C* were saponified by 1 hour of gentle ebullition. A low result was obtained with ethyl acetate when this time was reduced by one half.

Condenser *E* was then allowed to empty, cooling water was circulated in *B*, and the asbestos sheet over the flame was removed. These changes caused the excess methanol to distill from *C* and thereby made it possible to obtain a sharp end point, instead of a difficult one, when the residual aqueous liquor was titrated with the standard acid in presence of phenolphthalein. The sulfuric acid and the caustic soda were frequently standardized against each other and not more than 0.2 cc. of the alkali was utilized in blank experiments with the reagents. The difference between these blanks and the milliequivalents of alkali remaining in an actual experiment was accepted as equivalent to the amount of volatile acyl contained in the cellulose ester sample.

Table I. Acyl Analyses of Cellulose Mixed Esters[a]

Present Data		Eastman Kodak Company Data			
Apparent acetyl	Mean	Apparent acetyl	Acetyl	Propionyl	Butyryl
%	%	%	%	%	%
40.48, 40.21	40.35	40.2	29.3	13.9	0.6
42.87, 42.65	42.76	42.6	30.2	16.5	..
30.86, 30.62	30.74	30.7	19.8	14.6	..
40.37, 40.18	40.28	40.1	29.5	..	17.5
36.35, 36.39	36.37	36.3	21.2	..	24.9
32.80, 32.80	32.80	32.7	2.6	..	49.9

[a] Eastman Company sample numbers were from 102,429 to 102,434 in that order.

Results and Discussion

The determination in the form described above has been carried out more than 60 times with various cellulose esters. Very few of the duplicate estimations differed from the average acyl content by more than ±0.15 per cent; the great majority agreed to within 0.1 per cent and a considerable number to within 0.05 per cent. The dominant cause of deviation between duplicates was probably connected with the final titration, in which a buret reading to 0.1 cc. was used.

Pure ethyl acetate and pure, recrystallized sorbitol hexa-acetate gave data within 0.1 per cent of the calculated acetyl values, but doubt about the purity of cellulose ester samples made it more difficult to assess the accuracy of the method in these cases. The article by Gardner and Purves (*3*), however, contains examples in which the acetyl substitutions of a series of esters containing increasing amounts of the *p*-toluenesulfonyl radical were calculated from simultaneous equations involving both the acetyl and sulfur analyses. The acetyl substitutions found did not differ from their average value of 2.44 by more than ±0.03 in 21 out of 22 cases, and this error included those inherent in both the sulfur and the acetyl estimations.

Table I summarizes the results obtained with a series of cellulose acetate-propionates and acetate-butyrates kindly supplied, together with their analyses, by C. R. Fordyce, of the Eastman Kodak Company. Only three of the twelve independent estimations of "apparent acetyl" differed from the Eastman figures by more than 0.2 per cent and this divergence corresponded to a titration difference of about ±0.1 cc. in the experiments. The average values were about 0.1 per cent higher than the Eastman data, for reasons that are still obscure. These observations suggest that the present estimation is at least as accurate as the Ost technique, for which a precision of ±0.2 per cent is quoted (*4*), and may be applicable to cellulose butyrates, with which the Ost method often gives low results (*4*). The latter method also requires at least 7 hours of intermittent attention instead of the 5 or 6 hours demanded by the present one.

Although the procedure analyzed sorbitol hexa-acetate perfectly, it was not applicable to the acetates of reducing sugars because pure β-glucose penta-acetate analyzed 56.2, 59.4, 58.6, and 58.5 instead of the proper value of 55.2 per cent acetyl. The 6-chloro- or 6-iodo-6-desoxy-cellulose acetates also gave high acetyl data because traces of halogen-containing substances passed into the standard caustic soda solution. Corrections for this effect by estimating halogen in the titrated solution were not very successful, although proper allowance could be made in this way for samples containing 5 per cent of chlorine added as sodium chloride. A few analyses of cellulose acetate-nitrates also gave high results.

When both the "apparent acetyl" and the actual percentage of acyl groups in a mixed ester are known, an easy simultaneous equation makes it possible to calculate the individual amounts of the acyl substituents, provided only two are present. All the mixed esters in Table I except the first were accordingly deacylated by a modification of Zemplén's procedure (*9*) and the loss in weight of each sample was taken as equivalent to its original acyl content. Although the final data for acetyl, propionyl, and butyryl were always within 1 per cent of those in Table I, the calculation ignored the fact that the percentage of acyl found in this way differs from other acyl values in being based upon the equivalent weight of the radical *minus unity*. The agreement therefore owed much to the cancellation of errors and the method in its present state, although fairly rapid, is probably inferior in accuracy to those described by Malm and his collaborators (*5*, *6*).

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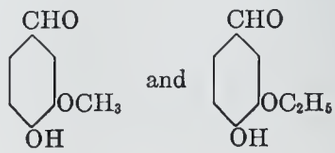
Contribution from the Research Laboratory of Organic Chemistry, Massachusetts Institute of Technology, No. 280.

Correction. In the article entitled "Detecting Adulteration of Ethylvanillin with Vanillin" [Ind. Eng. Chem., Anal. Ed., **15**, 268 (1943)] the structures of vanillin and ethylvanillin were incorrectly given. They should have been printed as:



Howard Nechamkin

Volumetric Determination of Bromide in Brines

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CERTAIN difficulties are encountered in the determination of bromide in salt brines containing much calcium and magnesium by the method of van der Meulen (*3*).

This involves the oxidation of bromide to bromate by means of hypochlorite at 80° to 90° C. in a solution buffered with boric acid and borate. The excess of hypochlorite is destroyed by adding an excess of hydrogen peroxide and the hydrogen peroxide is removed by boiling in the presence of osmium tetroxide as catalyst. The bromate is then determined iodometrically. The use of formate for the removal of the excess hypochlorite is also suggested (*4*).

D'Ans and Höfer (*1*) pointed out that the reaction is quantitative only within a definite pH range, but gave no numerical values. They suggested the use of sodium dihydrogen phosphate as a buffer, but this precipitates calcium and therefore cannot be used.

Kolthoff and Yutzy (*2*) used essentially the same procedure, but unfortunately gave no data on the limits for the pH. They used an ammonium molybdate solution as catalyst to hasten the reaction between potassium iodide and bromate, a suggestion already made by van der Meulen (*4*).

The difficulty with all the methods lies in the uncertainty in maintaining the solution at the correct pH when the bromide is oxidized to bromate. Since a solution containing no precipitate is preferable, phosphate buffers were not used in this work. The solutions were adjusted to the correct pH, using acetic acid–sodium acetate buffers. Experiments showed that the presence of acetate does not interfere. To find the limits of pH for the quantitative oxidation of bromide to bromate, samples containing a known amount of bromide and 5 grams of reagent sodium chloride were subjected to the procedure of Kolthoff and Yutzy except that an acetic acid–sodium acetate buffer was used. The results are shown in Table I. The pH was measured with a Beckman pH meter.

It is evident that the oxidation of bromide to bromate with hypochlorite is quantitative between pH 5.5 and 7.0.

The procedure was tested by analyzing synthetic brines, which were prepared as follows: 2.5 grams of anhydrous calcium chloride, purified by recrystallization from methanol and subsequent drying at 130° C., were dissolved in 25 ml. of water and 5 grams of reagent grade sodium chloride were added, followed by a known amount of potassium bromide solution. After 10 ml. of the hypochlorite solution were added, the pH was adjusted to between 6.0 and 6.5. Since the presence of hypochlorite interferes with the use of common acidimetric indicators, a small amount of zinc acetate was added to the solution. After some zinc hydroxide was precipitated glacial acetic acid was added dropwise until all zinc hydroxide was just dissolved. At this point the pH was between 6.3 and 6.8.

The solution was acidified with hydrochloric instead of sulfuric acid because of the presence of calcium. The results obtained are shown in Table II.

TABLE I. INFLUENCE OF pH ON OXIDATION OF BROMIDE TO BROMATE WITH HYPOCHLORITE

(Each sample contained 2.911 mg. of bromine as potassium bromide and 5 grams of reagent sodium chloride.)

pH	Bromide Found *Mg.*	Error *Mg.*	Average Error *Mg.*
5.0	2.84	−0.07	0.06
	2.88	−0.03	
	2.83	−0.08	
5.5	2.902	−0.009	0.012
	2.902	−0.009	
	2.928	+0.017	
6.0	2.911	0.000	0.002
	2.911	0.000	
	2.904	−0.007	
6.5	2.898	−0.013	0.008
	2.907	−0.004	
	2.904	−0.007	
7.0	2.902	−0.009	0.009
	2.906	−0.005	
	2.924	+0.013	
7.5	2.884	−0.027	0.028
	2.883	−0.028	
	2.882	−0.029	
8.0	2.68	−0.23	0.158
	2.82	−0.09	
	2.76	−0.15	
8.5	2.72	−0.19	0.179
	2.72	−0.19	
	2.76	−0.15	
9.0	Excess hypochlorite did not react with formate.		

TABLE II. DETERMINATION OF BROMIDE IN SYNTHETIC AND NATURAL BRINES

(Artificial brine contained 2.5 grams of anhydrous calcium chloride, 5 grams of sodium chloride, and 2.911 mg. of bromine as potassium bromide)

	Bromide Found *Mg.*	*Mg./l.*	Error *Mg.*
Artificial brine			
pH 6.25, glass electrode used	2.906	...	−0.005
	2.907	...	−0.004
pH 6.3 to 6.8, zinc acetate used	2.891	...	−0.020
	2.914	...	+0.003
	2.914	...	+0.003
Natural Michigan salt brines containing 175,000 p. p. m. of chloride, 10.00-ml. samples			
Brine A, pH 6.25	6.080	608.0	...
	6.081	608.1	...
Brine B, pH 6.25	7.486	748.6	...
	7.475	747.5	...

Special Solutions

Sodium Hypochlorite Solution. Dissolve 7.0 grams of reagent sodium hydroxide in 150 ml. of water, cool to room temperature, saturate with chlorine gas, and after the solution is distinctly yellowish pass air through for 10 minutes. Add 1.8 grams of reagent sodium hydroxide and dilute to 200 ml.

This solution contains enough free sodium hydroxide to be stable for a few days if kept cold. Sodium hypochlorite solutions with a pH below 12 are too unstable.

Sodium Formate Solution. 25 grams of C. P. sodium formate dissolved and diluted to 50 ml.

Procedure

Dilute or concentrate a sample containing no more than 13 mg. of bromide to 25 to 50 ml. in a 400-ml. beaker. If the solution is distinctly acid or alkaline, neutralize with acetic acid or sodium hydroxide solution. If there is not an excess of chloride in the sample, add 5 grams of reagent grade sodium chloride. After all solids have dissolved add 5 to 10 ml. of the sodium hypochlorite solution, depending on the amount of bromide present. Adjust the pH to between 6.0 and 6.5, using a glass electrode, by adding glacial acetic acid and, if necessary, sodium hydroxide solution. As alternative add a solution of 0.1 gram of zinc acetate in 5 ml. of water containing a few drops of acetic acid, and after obtaining a precipitate of zinc hydroxide add just enough glacial acetic acid dropwise to dissolve all zinc hydroxide. On swirling no zinc hydroxide should remain on the sides of the beaker.

Rinse down the sides of the beaker with water, heat over a small flame, and bring the solution to boiling in 5 to 10 minutes, then add 5 ml. of the sodium formate solution or 2.5 grams of solid C. P. sodium formate. This should cause an effervescence of carbon dioxide, indicating that a sufficient excess of the sodium hypochlorite was used. Rinse down the sides of the beaker and boil for a few seconds. Cool to room temperature, dilute to 150 to 200 ml., and add 5 ml. of a 20 per cent potassium iodide solution or 1 gram of solid potassium iodide. If free iodine is liberated at this point the sample must be discarded. Add 8 ml. of concentrated hydrochloric acid diluted to 30 ml. and 1 or 2 drops of 0.25 *M* ammonium molybdate solution. Titrate imme-

diately with a 0.01 N sodium thiosulfate solution, adding a little starch solution just before the end point, until the disappearance of the blue color.

Run a blank in the same manner, using the same amounts of reagents. The blank usually requires between 0.2 and 0.8 ml. of 0.01 N sodium thiosulfate solution.

One milliliter of 0.01 N thiosulfate solution corresponds to 0.00013319 gram of bromide.

The thiosulfate is conveniently standardized against analytical reagent potassium iodate.

Summary

An improved method of determining bromide in brines is described. The bromide is oxidized to bromate with hypochlorite at a pH of 5.5 to 7.0 which is adjusted by using zinc hydroxide as indicator or by using a glass electrode. After the removal of excess hypochlorite by formate the bromate is determined iodometrically. This method is rapid and is suitable for amounts of bromide up to about 13 mg. with an accuracy of about 2.5 parts in 1000.

Iodide and all oxidizing reagents that will liberate iodine from iodide in an aqueous solution interfere.

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(4) *Ibid.*, p. 238.

Determination of Water in Aniline by a Cloud Point Method

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A METHOD has been reported for determining water in aniline by means of the cloud point of a mixture of the aniline with rapeseed oil (*2*, *3*). Because rapeseed oil is imported and its sale is subject to governmental priorities, it seemed desirable to attempt to substitute an oil more easily available to American chemists, so that the use of the method (which is a good one) in the wartime production of aniline would not be hindered. It was found that a mixture of cottonseed oil and heavy mineral oil could be used satisfactorily instead of rapeseed oil, with some points of advantage for the mixed oil over the rapeseed oil. The method using mixed oil has a standard deviation from the true value for a single determination of ±0.006 per cent of water up to about 1.5 per cent of water, and ±0.009 per cent from 1.5 to 4.2 per cent of water. The standard deviation using rapeseed oil was found to be ±0.015 per cent of water up to a water content of 2 per cent.

Reagents

Cottonseed Oil. The commercial "cooking oil" first used had a specific gravity (25° C./25° C.) of 0.9172 and a refractive index (n_D^{20} ° C.) of 1.47324, and the insoluble fatty acids obtained upon saponification had a freezing point of 31.9° C. The oil gave a positive response to the Halphen test for cottonseed oil (red coloration upon heating in carbon disulfide with amyl alcohol and sulfur). Wesson oil, a brand of cottonseed oil, which was also used, had a specific gravity (25° C./25° C.) of 0.9171.

Mineral Oil. The mineral oil first used was a heavy white oil that had a specific gravity (25° C./25° C.) of 0.8756 and a refractive index (n_D^{20} ° C.) of 1.47916. Nujol, a brand of medicinal mineral oil, which was also used, had a specific gravity (25° C./25° C.) of 0.8757.

Mixed Oil. Five parts by volume of cottonseed oil and one part of mineral oil were used with the slight adjustment described below.

Apparatus and Procedure

All apparatus must be dried by heating and cooling in a desiccator, by a stream of dried air, or, for the aniline pipet, by washing several times with the aniline sample to be analyzed.

With a 5-ml. Mohr pipet, transfer 3.5 ml. of the mixed oil to a test tube (2.5 × 15 cm., 1 by 6 inches) and then add (25-ml. Mohr pipet) 20 ml. of the aniline. Insert into the test tube a two-hole cork stopper containing in one hole a thermometer and in the other a stirrer with a loop to encircle the thermometer. (Any efficient stirrer can be used.) The thermometer may be of any type, but it should be calibrated in tenths of a degree centigrade and the temperature should be capable of being estimated to at least 0.05° C. There should be about 5-mm. clearance between the bottom of the test tube and the bottom of the thermometer bulb. When 20° range thermometers are used, two are necessary, one covering the range 30° to 50° C. for water up to about 2.5 per cent, and the other 40° to 60° C. for water from 2.5 to about 4.5 per cent.

With constant stirring of its contents, the tube is dipped into a warm water bath until the opaque emulsion just changes to a slightly turbid liquid. It is immediately removed from the bath in order to avoid too high a rise in temperature, and its contents, which are now clear, are stirred with long even strokes (about 50 to 80 per minute) until the cloud point is reached. This is recorded to the nearest 0.05° C. About 0.3° to 0.4° C. above the cloud point, a slight turbidity develops, which increases gradually upon cooling, giving an indication of the approach of the cloud point. Then suddenly, within a temperature drop of less than 0.05° C., the mixture becomes milky and practically opaque. This constitutes the cloud point.

The percentage of water may be calculated from the linear equation:

$$\text{Per cent water by weight} = \frac{\text{cloud point found (° C. corr.)} - \text{cloud point of dry aniline (° C. corr.)}}{\text{mean variation of cloud point for 1 per cent variation in water content}}$$

Substituting the proper constants, for cloud point values up to about 42.60° C. (1.15 per cent water) the formula becomes:

$$\text{Per cent water by weight} = \frac{\text{cloud point found (° C. corr.)} - 36.80}{5.043}$$

For values of the cloud point from 42.60° to 58.75° C. (4.2 per cent water) the value of the denominator changes and the equation becomes:

$$\text{Per cent water by weight} = \frac{42.60 - 36.80}{5.043} + \frac{\text{cloud point found (° C. corr.)} - 42.60}{5.263} = 1.15 + \frac{\text{cloud point found (° C. corr.)} - 42.60}{5.263}$$

Duplication of Mixed Oils

When a fresh batch of mixed oil is made up, it can be adjusted by adding cottonseed or mineral oil, so that dry aniline will have a cloud point of 36.80° C. within the normal variation. The formulas could then be used without change, but it is advisable to check them with a few samples of aniline of known water content. About 7 or 8 ml. of mineral oil added to 1 liter of 5 to 1 mixed oil raise the cloud point by about 1°, whereas about 40 ml. of cottonseed oil lower the point by the same amount. It is thus easier to adjust finally by adding cottonseed oil rather than mineral oil.

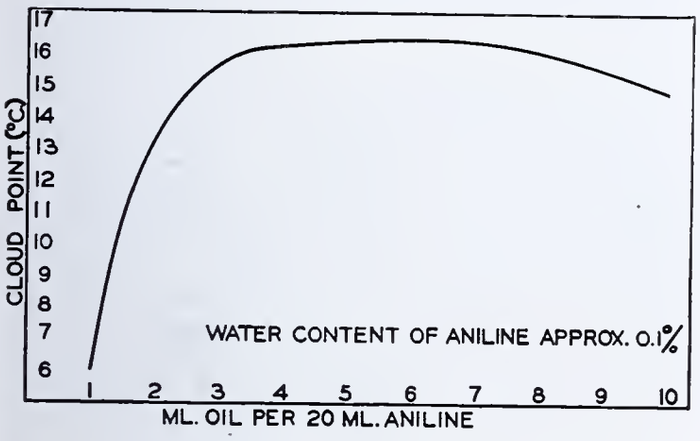

FIGURE 1. VARIATION OF CLOUD POINT WITH CHANGING RATIO OF COTTONSEED OIL TO ANILINE

In this way it is possible to adjust the proportions of cottonseed and mineral oils of different origin so that the given formulas may be used, but this adjustment must be made by trial and error. Merely to mix cottonseed and mineral oils in the same proportions will not necessarily give an oil which will fit the formulas. For instance, before any adjustment, two samples of mixed oil (made from cottonseed and mineral oils from different sources, but mixed in the same proportions as exactly as possible) gave, with the same sample of aniline, cloud points of 39.70° and 36.90° C., respectively.

Fairly dry oils should be used, but small variations in their water content are of slight influence. If water is present, it will effect the initial cloud point of the mixed oil, but since the latter is finally adjusted to a definite value, the adjustment automatically corrects for the water present. This would obviously not be possible with a single oil such as rapeseed oil.

A mixed oil was prepared from Wesson oil and Nujol, in order to determine whether adjustments of a fresh batch of oil, made with different samples of cottonseed oil and mineral oil, to a 36.80° C. cloud point for dry aniline would maintain the correctness of the formulas. Ten known aniline solutions containing from 0.00 to 1.18 per cent by weight of water were examined. The values found by using the formulas agreed with the known values as closely as the known accuracy of the method would lead one to expect.

Precision and Accuracy

The formulas given above are based upon a plot of a total of 51 values for cloud points obtained with aniline samples of known water content.

The precision of the values obtained is less for samples with more than 1.15 per cent of water than for those with less water. Using solutions of water in aniline ranging from 0.000 to 1.199 per cent of water, the cloud points deviated from those calculated from the formula by from +0.07° to −0.05° C. for 34 values. The standard deviation of a single value from the arithmetic mean was ±0.03° C. (equivalent to ±0.006 per cent of water). (The standard deviation is the square root of the arithmetic mean of the squares of the deviations of the individual values from their arithmetic mean. It represents the range above and below the arithmetic mean within which about 68 per cent of the determined single values will lie. By determining more than one value, the standard deviation of the mean will be that of a single value divided by the square root of the number of values used in obtaining the mean value.)

In the range 1.514 to 4.230 per cent of water, the cloud points deviated from those calculated from the formula by from +0.08° to −0.07° C. for 17 values. The standard deviation was ±0.046° C. (equivalent to ±0.009 per cent of water). It is obvious that the precision of the values should decrease with an increase in the difference between the cloud point and room temperature, as occurs with the higher values, because of more rapid cooling.

If the aniline used for determining the constants of the equations can be shown to be free of water in so far as this can be proved, considering the precision of the method, the precision will then be synonymous with the accuracy. Mere distillation in a well-dried system and collection of a middle cut of constant boiling point was unsatisfactory, yielding aniline which had 0.02 per cent of water. The aniline which was finally used was obtained by distilling 2.5 liters of aniline, collecting 21 cuts, and determining cloud points on alternate cuts. It was assumed that constancy of the cloud point would indicate a dry aniline. This assumption was based on the high sensitivity of the method to the presence of small amounts of water. (It might, of course, be sufficient to dry the aniline merely by an efficient desiccant.) After the first cut of 350 ml. (cloud point 37.30° C.) the cloud points of all the other cuts (1965 ml.) varied between 36.77° and 36.87° C. for ten values, with no trend toward lower cloud points in the later cuts. The mean value was 36.80° C. with a standard deviation of a single value from the mean of ±0.048° C. Since 36.80° C. is a mean of ten values, its standard deviation would be $\frac{\pm 0.048°\ \text{C.}}{\sqrt{10}}$, or ±0.015° C., which is less than the limit of reading the thermometer. Consequently, the value taken for dry aniline is of sufficient precision to indicate that the method is as accurate as it is precise.

Search for Most Suitable Oil

Before deciding upon a mixed cottonseed-mineral oil to replace rapeseed oil, other possibilities were considered. Mineral oil alone was not satisfactory. When as little as 1 ml. was used with 25 ml. of stock aniline containing no added water, the mixture remained cloudy even in a boiling water bath. Castor oil alone was too viscous for satisfactory use; besides, mixtures with the stock aniline containing 1 ml. of the oil to 25 ml. of aniline or equal proportions of oil and aniline did not become turbid even in an ice bath. A mixture of 5 ml. of castor oil and 1 ml. of mineral oil with 25 ml. of aniline gave a fairly reproducible cloud point at

about 53.5° C., but the point was not so sharp as with rapeseed oil and, in addition, oily droplets persisted even above the temperature at which the main turbidity had vanished. Additions of 1 and 2 per cent of water caused increases of about 4.5° and 7.5°, respectively, in the cloud point, but the oily droplets still persisted.

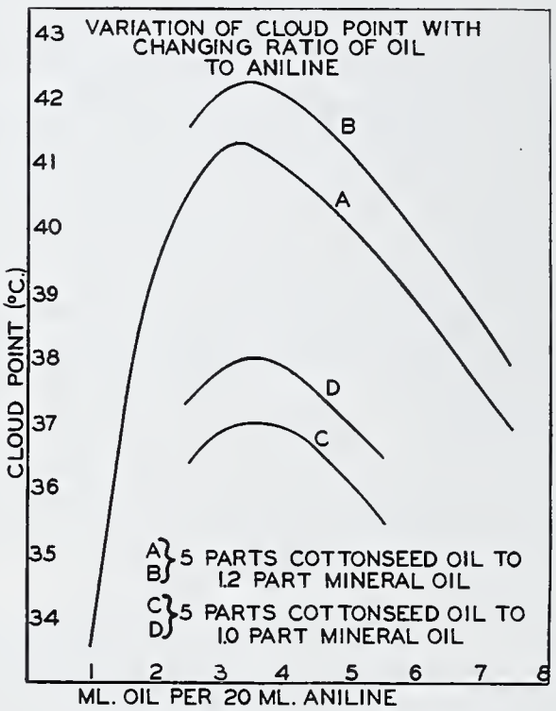

Figure 2. Cottonseed-Mineral Oil Mixtures

Jansen and Schut (*3*) studied the cloud points of aniline containing water in solution, by means of rapeseed, soy, arachis, and maize oils added in the proportion of 1 part of oil to 4 parts of aniline (by weight). They reported cloud points for dry aniline with these oils as 37.6°, 8.7°, 26.2°, and 11° C., respectively.

Before fixing the proportions of mixed oil as 5 parts by volume of cottonseed oil to 1 part of mineral oil, the cloud points were determined with cottonseed oil alone added to aniline in various proportions. The oil was unsuitable in any proportion which was tried, because the cloud points obtained with it were not only too low for manipulative convenience, but could not be obtained with sufficient precision. Figure 1 illustrates the variations in cloud points of a stock sample of aniline with changes in the proportion of cottonseed oil used. Even the highest cloud point obtained is below the usual room temperature. The use of this oil would, therefore, necessitate a cooling bath that would have to be regulated to within a few degrees of the expected cloud point. Even then the precision was actually considerably poorer than with the mixed oil.

On the basis of the behavior of the aforementioned oils and with a consideration of their availability, mixtures of mineral oil and cottonseed oil were tried.

Figure 2 gives the variation in the cloud point with a changing ratio of mixed oil to aniline for a mixture of 5 parts (by volume) of cottonseed oil to 1.2 parts of mineral oil (curves A and B) and for another mixture of 5 parts of cottonseed oil to 1.0 part of mineral oil (curves C and D). A and C are for stock aniline, and B and D are for stock aniline with 0.2 per cent of added water. With both oil mixtures the cloud point reaches a maximum with 3.5 ml. of oil for 20 ml. of aniline, but with the 5 to 1.0 mixture the slope of the curve changes more slowly near the maximum than with the 5 to 1.2 mixtures. Because of this, the 5 to 1 mixture, when used in the ratio of 3.5 ml. of the mixture to 20 ml. of aniline, would suffer less change in cloud point because of small variations in the ratio of mixed oil to aniline, and so would be preferable to the 5 to 1.2 mixture.

Comparison of Rapeseed Oil and Mixed Oil Procedures

The use of the mixed oil instead of rapeseed oil has several advantages.

With rapeseed oil the transition from a clear to a cloudy solution is abrupt, so that the analyst has no warning of the approach of the cloud point. With the mixed oil a region of about 0.3° to 0.5° C. occurs upon cooling, in which the mixture gradually assumes a turbid appearance, which is followed by the abrupt formation of a distinct opacity. The latter is taken as the cloud point and is reproducible. Dolique (*1*) has reported that a similar phenomenon takes place upon adding various substances to the phenol-water system when the added substances contain impurities. He considers this behavior to be characteristic of quaternary systems for low concentrations of the fourth component. In the analysis for water in aniline the water would serve as the fourth component present in low concentration.

Another advantage lies in the fact that with the mixed oil there seems to be less tendency toward a loss of moisture during the heating preliminary to determining the cloud [illegible] The loss of water with both oils is, of course, greater [illegible] than for low concentrations of water. For water [illegible]centrations from 0 to about 1 per cent, reheating the aniline-oil mixture in the manner described for the test caused the cloud point to drop about 0.05° C. with rapeseed oil, and from 0° to 0.05° C. with the mixed oil. For aniline containing from 1 to about 3 per cent of water, with the use of rapeseed oil the cloud point suffered a change of 0.2° to 0.4°, whereas with the mixed oil it changed by from 0° to 0.15°. For a sample of aniline containing 4.2 per cent of water, with rapeseed oil the change was 0.8° to 1.0°, whereas with the mixed oil the change was only 0.20° to 0.25°. This would indicate that the use of the mixed oil might offer less liability of error because of change in composition during the preparation of the mixture for the test.

Still another advantage of the mixed oil over the rapeseed oil is the possibility of adjusting any batch of oil to the value obtained with the previous batch, regardless of the differences in moisture content of the oil. With rapeseed oil, an entire new calibration might have to be made with every new batch of oil. For instance, the authors found when using a commercial rapeseed oil that the cloud points from 0 to 2 per cent of water were 35.90° to 47.75° C. corrected, respectively, about 1.4° C. lower than the corresponding values reported by Griswold (*2*). The slopes of the lines representing the two sets of data were, however, identical.

The mixed oil also appeared to have some advantage in precision. Nineteen cloud point values were obtained with rapeseed oil on solutions of water in aniline ranging from 0 to 2.2 per cent by weight. The values deviated from the best possible mean straight line by from +0.15° to −0.15° C. The standard deviation was ±0.087° C., equivalent to ±0.015 per cent of water. The standard deviation calculated from Griswold's data is ±0.027 per cent by weight of water for values up to about 2 per cent of water.

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Determination of the Saponification Number of Fats and Oils

Elimination of the Blank

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A "DOUBLE-indicator method" has been developed for determining the saponification number of fats and oils. For almost all oils this method gives excellent checks with the standard method and requires less time, reagents, and apparatus.

Figure 1 represents the potentiometric titration of the solution obtained by saponifying 5 grams of oil or fat with 50 ml. of alcoholic potassium hydroxide according to the standard procedure (*1*). The acid added up to the first point of inflection is used to neutralize the excess potassium hydroxide. The acid added between the two points of inflection is used for the reaction

$$RCOO^- + K^+ + H^+ + Cl^- \longrightarrow RCOOH + K^+ + Cl^-$$

Some of the fatty acids formed in this part of the [illegible] separate as a solid or liquid phase. The acid added be[illegible] the two jumps is equivalent to the soap formed or to the potassium hydroxide that reacted with the oil during the saponification. Therefore, the saponification number of the oil can be calculated from the weight of oil taken, the volume of acid used between the two jumps, and the concentration of this acid. The volume of alcoholic potassium hydroxide and its concentration are not required for the calculation, and the blank titration is eliminated.

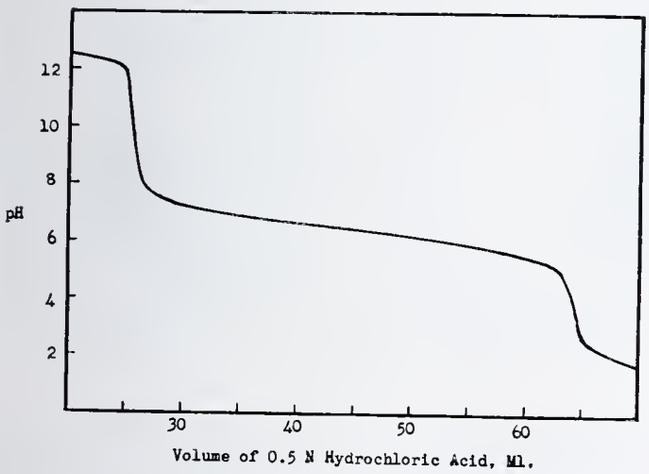

FIGURE 1. TITRATION GRAPH OF SAPONIFIED CORN OIL

However, the slope of the graph at the second equivalence point is ordinarily not great enough to permit an accurate determination of this point either with the potentiometer or with a suitable indicator. This slope is given by the equation (*2*, *3*)

$$\frac{d\text{pH}}{dv} \cong -0.22\,N\sqrt{\frac{1}{KLV}}$$

where v is the buret reading, N the normality of the hydrochloric acid, K the ionization constant of the fatty acid, V the total volume of the solution, and L the number of millimoles of fatty acid in solution at the equivalence point. An accurate solution of this equation is not practical because the titration deals with a mixture of fatty acids rather than with a single acid and because the ionization constants of the acids in the mixture of alcohol and water are not known.

Nevertheless, the equation indicates that the slope can be increased (arithmetically) by decreasing the quantity of fatty acid dissolved in the alcohol-water mixture. This is readily accomplished by the addition of 10 ml. of benzene, which extracts most of the fatty acids from the aqueous layer. This removal of the fatty acid also raises the pH of the equivalence point. For example, in the titration of saponified corn oil without the addition of benzene, the second point of inflection occurred at a pH of 3.76 and had a slope of −2.1 pH units per ml.; in another titration performed by the same procedure except for the addition of 10 ml. of benzene, the pH at the second point of inflection was 4.31 and the slope was −6.9.

Reagents

Alcoholic potassium hydroxide is prepared according to the standard procedure, the alcohol being previously distilled from a mixture of potassium hydroxide and aluminum (*1*). The solution is filtered to remove potassium carbonate. The storage bottle is fitted with a two-hole rubber stopper which carries an Ascarite tube and a siphon. Thus the solution is effectively protected against the absorption of carbon dioxide.

Bromophenol blue (0.010 *M*) is prepared by mixing 1.3 grams of the indicator with 2.0 millimoles of sodium hydroxide and diluting with water to 200 ml.

Procedures

POTENTIOMETRIC METHOD. Five grams of oil are saponified according to the standard method (*1*), except that the volume of alcoholic potassium hydroxide is measured only roughly and a U-shaped Ascarite tube is attached to the upper end of the reflux condenser. This tube contains Ascarite in only one side, and the other side (inverted) is attached to the reflux condenser. Thus there is no possibility that any Ascarite will contaminate the solution.

After saponification, the solution is cooled to room temperature, or slightly above room temperature if necessary to prevent the crystallization of the soap. A cork stopper fitted with glass and calomel electrodes is placed in the neck of the flask. The solution is titrated with standard 0.5 *N* hydrochloric acid, the pH readings being taken on a Beckman pH meter, laboratory model. In the vicinity of the first equivalence point, the acid is added in increments of 0.10 ml. After the first equivalence point, 10 ml. of benzene are added, and the titration is continued. Near the second equivalence point, increments of 0.10 ml. are again added, and the flask is swirled vigorously after each addition to hasten the extraction of the liberated fatty acids by the benzene.

The inflection points are located by calculating the second differentials of the pH with respect to the buret reading, $\Delta^2\text{pH}/\Delta v^2$, and interpolating to find the buret reading at which this differential is zero. The slopes are considered to be ten times the maximum increment in pH.

DOUBLE-INDICATOR METHOD. The oil is saponified as in the potentiometric method. After cooling, 17 drops (0.3 ml.) of 1 per cent alcoholic phenolphthalein are added. Then 0.5 *N* hydrochloric acid is added until the color of the indicator dis-

appears. The volume of acid used to reach this end point need not be measured. Then 3 drops (0.2 ml.) of 0.010 *M* bromophenol blue and 10 ml. of benzene are added, the buret is refilled to the zero mark with standard 0.5 *N* hydrochloric acid, and the solution is titrated to a green color.

This end point is sharp. The solution is distinctly blue a drop or two before the end point, and distinctly yellow a drop or two beyond. Nevertheless a few cautions may be helpful. The solution ordinarily turns yellow several drops before the end point is reached. Then on agitation, the color changes to blue again as the fatty acids are extracted by the benzene. The end point is a green color that is not changed to blue by further swirling of the flask. The benzene also extracts the coloring matter (usually yellow) of the oils. Upon agitation of the flask before the end point is reached, the emulsion of the yellow benzene in the blue aqueous phase sometimes gives the appearance of the green end point. Fortunately, the two phases separate quickly when the agitation is stopped, and the color of the aqueous phase can be observed.

Table I. Summary of Potentiometric Titrations

Oil	First Jump pH	First Jump Slope	Second Jump pH	Second Jump Slope
Butter fat	10.70	9.6	3.89	2.9
Castor oil	10.03	9.6	4.49	7.1
Castor oil, acetylated	11.14	9.7	3.27	0.8
Cocoa butter	10.66	15.1	4.28	6.1
Coconut oil	10.62	9.4	4.03	3.9
Cod liver oil	10.50	9.7	4.45	6.8
Corn oil	10.13	8.5	4.31	6.9
Lard oil	10.49	8.7	4.46	6.0
Linseed oil	9.85	8.1	4.38	6.8
Neat's-foot oil	9.86	8.8	4.41	4.4
Olive oil	10.35	8.3	4.55	5.5
Palm oil[a]	10.00	5.5	3.33	0.8
Rapeseed oil	10.15	7.4	4.61	4.8
Tung oil	10.28	6.2	4.42	4.6

[a] See discussion.

Results

The pH values and the slopes at the two equivalence points for the titrations of various oils are recorded in Table I. Ten milliliters of benzene were added after the first equivalence point in all these titrations.

A comparison of the results obtained by the recommended methods with those of the standard method (*1*) is given in Table II.

Discussion

It is well known that the glass electrode fails to check the hydrogen electrode in solutions of high pH and also in solutions containing a large concentration of alcohol. Therefore the values read on the scale of the Beckman pH meter in this work should not be interpreted as exponents of hydrogen-ion activity or concentration. The uncertainty of the liquid-junction potential between the aqueous solution in the calomel electrode and the mixed solvent adds further doubt to the meaning of the pH values. Nevertheless, these values were perfectly reproducible and changed on addition of acid or base in the expected direction. They may be used to compare the acidity of two solutions that have approximately the same ratios of alcohol to water.

Varying quantities of phenolphthalein were added to some of the solutions that were titrated potentiometrically. It was found that the disappearance of the indicator color coincided best with the inflection point when 17 drops of 1 per cent phenolphthalein were added. Therefore this quantity was used in the double-indicator method.

Table I reveals that for all the oils studied, except acetylated castor oil and palm oil, the second inflection point occurs near a pH of 4.3. Bromophenol blue was chosen as the indicator for this end point because it shows a sharp color change at a pH of 4.3 in the mixed solvent.

Table II reveals that the recommended methods give excellent checks with the standard method for all the oils studied except acetylated castor oil and palm oil. The failure of the recommended methods with acetylated oils is to be expected because the benzene fails to extract acetic acid from the aqueous phase. This results in a low pH at the second equivalence point, a small slope of the graph at this point, and hence an unsatisfactory end point with the indicator.

The behavior of the sample of palm oil is more puzzling. The high saponification number obtained by the standard method indicates that the oil was not pure and that palm nut oil was a likely adulterant. The low pH and small slope at the second equivalence point indicate the presence of fatty acids of very low molecular weight.

The potentiometric method is time-consuming and is recommended only for highly colored oils. The double-indicator method, however, gives accurate results and requires less time, reagents, and apparatus than the standard method.

The double-indicator should be more easily adaptable to microprocedures than the standard method. In any micromodification of the standard procedure, it would be necessary either to measure accurately a very small volume of alcoholic potassium hydroxide or to use a very dilute solution of this reagent. In the first alternative, difficulty would be encountered because of the volatility and large expansion coefficient of the alcohol. In the second alternative, the time of saponification would be prolonged. In the case of the double-indicator method, a small volume of fairly concentrated alcoholic potassium hydroxide could be used without the necessity for accurate measurement or control of this volume.

Table II. Comparison of Results by Various Methods

Oil	Standard Method Saponification No.	Potentiometric Method Saponification no.	Potentiometric Method Difference	Double-Indicator Method Saponification no.	Double-Indicator Method Difference
Butter fat	224.1	224.2	+0.1	223.8	−0.3
Castor oil	181.5	181.2	−0.3	181.0	−0.5
Castor oil, acetylated	388.4	390.1	+1.7[a]	[b]	...
Cocoa butter	194.6	194.9	+0.3	194.7	+0.1
Coconut oil	258.6	258.6	0.0	258.6	0.0
Cod liver oil	188.0	188.4	+0.4	188.3	+0.3
Corn oil	191.6	191.5	−0.1	191.8	+0.2
Lard oil	195.5	195.5	0.0	195.7	+0.2
Linseed oil	187.0	186.7	−0.3	186.6	−0.4
Neat's-foot oil	186.7	186.7	0.0	187.1	+0.4
Olive oil	191.5	191.4	−0.1	192.3	+0.8
Palm oil	238.0	237.1	−0.9[a]	[b]	...
Rapeseed oil	174.3	174.5	+0.2	174.4	+0.1
Tung oil	193.5	193.2	−0.3	192.9	−0.6
Mean (signs disregarded)			±0.2		±0.3

[a] Omitted in calculating mean.
[b] Method not applicable to these oils.

When several saponification numbers are to be run, the following modification of the double-indicator method may be advantageous:

Pipet 50.00 ml. of alcoholic potassium hydroxide into each of the flasks containing the weighed samples of oils. After saponification, titrate two or three samples by the double-indicator method, but measure the volume of standard hydrochloric acid used to reach both the first and the second end points. The total volume of acid is the same as the blank titration of the standard method. Then the remaining samples need to be titrated only to the phenolphthalein end point.

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Colorimetric Determination of Hydrogen Peroxide

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DURING a study of the effect of humidity on the production of ozone from air by the action of the silent electric discharge, qualitative tests revealed the formation of hydrogen peroxide. Attempts at quantitative estimation by means of the commonly employed volumetric methods and by those few colorimetric procedures (*3*, *4*) reported in the literature were not entirely satisfactory. It was therefore necessary to develop a method which would be sensitive to very small amounts of peroxide. This procedure, in comparison with other methods based on the same chemical reaction, has the advantage of yielding a straight-line calibration curve over the concentration range specified.

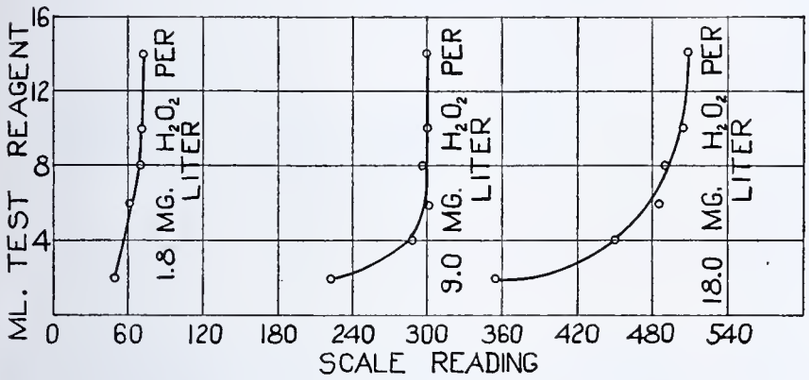

FIGURE 1. EFFECT OF VARYING QUANTITIES OF TEST REAGENT ON COLOR INTENSITY

K. & S. colorimeter, filter 40, cell depth 40 mm.

Principle of Method

The method is based on photoelectric measurement of the color intensities of hydrogen peroxide solutions treated with titanium sulfate reagent. The yellow color produced in the reaction was first observed by Schonn (*5*) and according to Treadwell and Hall (*6*) is due to the formation of pertitanic acid. The reaction equation is usually written

$$Ti^{++++} + H_2O_2 + 2H_2O = H_2TiO_4 + 4H^+$$

although some chemists prefer to write the formula of pertitanic acid as $TiO_2.H_2O_2$, showing a true peroxide structure. It was Jackson (*2*) who pointed out the quantitative possibilities. Richardson (*4*) and Knecht (*3*) developed methods in which colors were compared visually. More recently Allsop (*1*) reported on a photoelectric method which is discussed in greater detail below.

Reagents and Solutions

All reagents were of the purest grade obtainable.

The titanium sulfate test reagent was prepared by digesting 1 gram of anhydrous titanium dioxide (Schering-Kahlbaum) with 100 ml. of sulfuric acid (specific gravity 1.84) for 15 to 16 hours on a sand bath at a temperature of 150° C. The solution was cooled, diluted with 4 parts (by volume) of distilled water, and filtered through an asbestos mat prior to use. Preliminary experiments in which varying quantities of reagent, ranging from 2 to 50 ml., were diluted to 100 ml. with distilled water indicated no color or turbidity to be present.

A standard hydrogen peroxide solution was made by diluting 20 ml. of 30 per cent hydrogen peroxide solution to 1 liter with distilled water and standardized by titration with permanganate.

Apparatus

The photoelectric colorimeter was a Klett-Summerson Model 900–3 in which was used a KS-40 filter (transmission limits 380 to 430 millimicrons). An unusual feature of this instrument is the scale on which readings are made. This scale is logarithmic and has been designed so that under certain specified conditions the scale readings are a measure of and are proportional to the optical density of the colored solution as determined by the photoelectric cell. Since the optical density is theoretically proportional to the concentration of the colored substance, the scale readings are likewise proportional to the concentration under the specified conditions.

Optimum Quantity of Test Reagent for Maximum Color Development

To ascertain whether the intensity of color is a function of the quantity of titanium sulfate used, varying quantities of the test reagent were added to known quantities of hydrogen peroxide solution contained in a series of 100-ml. Nessler tubes. All solutions were then diluted to 100-ml. volume with distilled water and the intensity of color was measured by transferring to the solution cell of the colorimeter. In all, three such experiments were made in which concentrations of 18, 9, and 1.8 mg. of hydrogen peroxide per liter were used. Cell depth was 40 mm. Results are shown graphically in Figure 1.

It is evident that 1 volume of titanium sulfate reagent per 10 volumes of peroxide solution is required for maximum color development. As far as could be ascertained, the color is developed instantly and remains stable for at least 6 hours. In view of this finding, 10 ml. of reagent were used in all subsequent experiments.

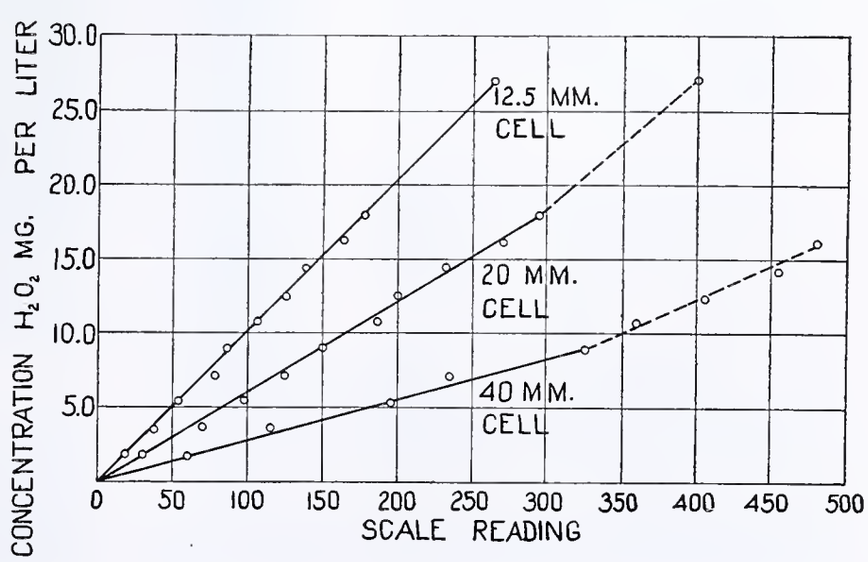

FIGURE 2. CONFORMITY TO BEER'S LAW

Table I. Accuracy of Method

Scale Reading *Divisions*	(12.5-mm. cell) Concentration of H_2O_2 *Mg./100 ml.* Actual	Calculated	Deviation *Mg./100 ml.*
18	0.18	0.18	0.00
38	0.36	0.39	+0.03
54	0.54	0.55	+0.01
78	0.72	0.80	+0.08
86	0.90	0.88	−0.02
106	1.08	1.08	0.00
125	1.26	1.27	+0.01
139	1.44	1.42	−0.02
164	1.62	1.67	+0.05
177	1.80	1.80	0.00
263	2.70	2.68	−0.02
			Av. ±0.03

Conformity to Beer's Law

A series of solutions, containing from 1.8 to 27 mg. of hydrogen peroxide per liter, was prepared by placing the appropriate quantity of peroxide in 100-ml. Nessler tubes, adding 10 ml. of titanium sulfate reagent to each tube, and diluting to the mark with distilled water. After thorough mixing the solutions were transferred to the colorimeter cell. Color intensity was measured using three different cell depths: 40, 20, and 12.5 mm.

That Beer's law is followed by the color system is evident from the fact that a straight line is obtained when the scale readings are plotted against the hydrogen peroxide concentration (Figure 2). The deviation from the straight-line relationship for the higher color intensities (scale readings above 300) observed in the cases of the 20- and 40-mm. cell depths is probably due to the fact that a relatively wide band-pass filter was used. The use of a smaller cell, 12.5 mm., eliminates this deviation and increases the effective range of the method. In view of this, in order to obtain conformity to Beer's law throughout any given range of concentrations, it is necessary that the cell depth be adjusted to the colorimetric system.

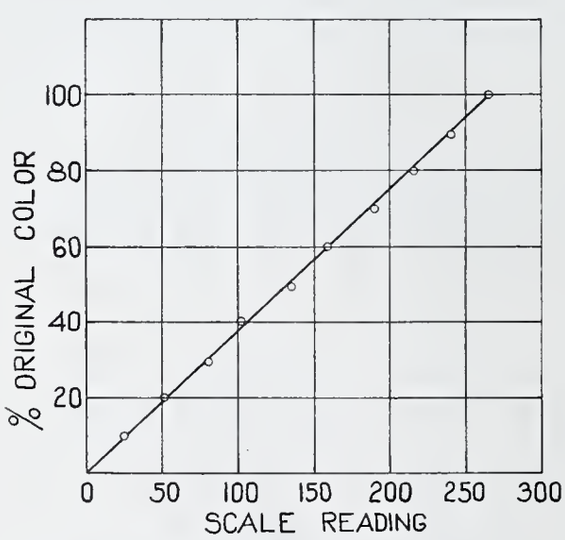

Figure 3. Effect of Dilution on Color Intensity

Figure 3 shows the results of tests made to determine whether relatively dark solutions could be diluted and still have the resultant color system conform to Beer's law. It is obvious that this can be done.

Allsop (*1*) reported on a photoelectric method which, although similar in some respects to the one described herein, does not give a linear calibration curve over the entire concentration range nor does it cover the same range of concentrations. Using a Hilger absorptiometer equipped with a 4-cm. cell and a blue filter giving maximum transmission at 470 millimicrons, he measured the optical densities of solutions containing varying amounts of hydrogen peroxide treated with titanium sulfate. For purposes of comparison, his data are shown graphically in Figure 4, together with the results obtained by the author. The difference in the linear character of the two curves is obvious.

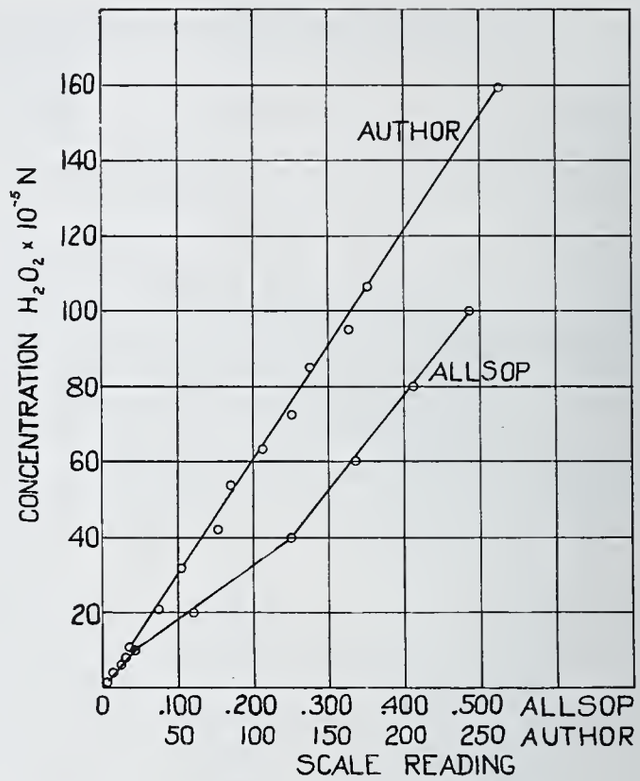

Figure 4. Comparison of Allsop's and Author's Data

The accuracy with which hydrogen peroxide can be determined with this method is shown in Table I. With the 12.5-mm. cell it is possible to determine hydrogen peroxide in concentrations ranging from approximately 0.2 to 3.0 mg. per 100 ml. with an average deviation of ±0.03 mg. per 100 ml.

Summary and Conclusions

A rapid and accurate colorimetric method for the determination of hydrogen peroxide is described. Beer's law is valid for measurements at 380 to 430 millimicrons. However, in order to obtain conformity to Beer's law throughout any given range of concentration, it is necessary that the cell depth be adjusted to the colorimetric system. A 12.5-mm. cell increases the effective range of the method, permitting determination of hydrogen peroxide concentrations ranging from approximately 0.2 to 3.0 mg. per 100 ml. of solution with an average deviation of 0.03. Relatively dark solutions can be diluted without invalidating Beer's law. For maximum color development 1 volume of test reagent per 10 of solution is required.

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Current Methods of Measuring Foam

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WHEN methods of foam measurement were previously considered, an initial classification was based on the obvious differences between "static" and "dynamic" methods. Static methods are those in which measurements are made after the foam has been produced, with no reference to the manner of its production. In dynamic methods measurements are made while the foam is being formed, usually by a current of gas passing through the liquid.

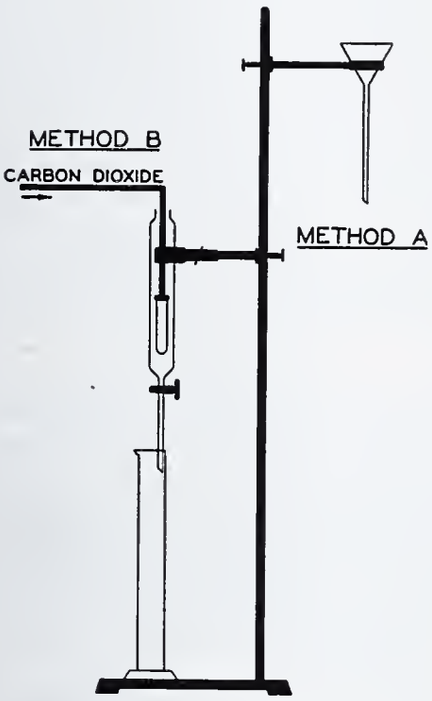

FIGURE 1. ROSS AND CLARK STATIC FOAM APPARATUS
Method A, applicable to carbonated liquids
Method B, applicable to uncarbonated liquids

It has already been shown that results obtained by the various methods do not necessarily agree (*5*). It is not generally possible, when dealing with a property of matter so complex as the foaminess of a liquid, to make classifications that can be universally applied. In the present case it is recognized that static and dynamic methods represent only an empirical distinction and that there exist more fundamental theoretical considerations upon which a final classification of methods must be based. Meanwhile the two terms are of value in providing a method of approach to the problem of foam measurement, especially with a view to unraveling the complex mass of results and conclusions contained in the literature on the subject.

It is proposed in the present paper to examine some of the recently published methods of both types, to obtain evidence of whatever correlations may exist, and to establish if possible a better understanding of the factors involved.

Static Methods Involving Drainage

Static methods usually consist of forming a foam, in any one of a number of possible ways, and observing the rate at which liquid drains or collects from it, beginning the observations some time after cessation of foam formation. It has been discovered experimentally that for many foams the rate of drainage of liquid obeys the equation

$$V = V_0 e^{-kt}$$

$$\text{or} \quad \ln V_0/V = kt$$

where V = volume of liquid in the foam after time t, V_0 = volume of liquid in the foam when $t = 0$, t = time, and k is a constant characteristic of each liquid from which the foam is formed. This logarithmic equation has been found to hold for such diverse substances as beer (*4*), wine (*1*), saponin (*2*), Aerosol OT (*15*), and lauryl sulfonic acid (*15*). It would therefore appear to be a general law for the behavior of foams. Deviations are known to exist—e. g., foams of egg albumin (*5*)—but they can be explained on the basis of specific considerations for the case in point.

Using this mathematical relation, it was possible to establish a unit of foaminess for all static foams (*5*, *16*), which can be applied regardless of the way in which the foam is produced and independently of many empirical restrictions of former methods.

Figure 1 illustrates the Ross and Clark static method for both carbonated (method A) and uncarbonated liquids (method B). The foam is produced in the former case by pouring the liquid from a height and in the latter case by bubbling a gas through it. In both cases the same value for the foaminess was obtained, using the reciprocal of the constant of the exponential equation as a unit of foaminess. A modification of method B, capable of greater accuracy, is illustrated by Figure 2. This is the Stiepel foam meter and can be used to measure not only the rate of drainage of the liquid but also the volume of foam produced. In this way it can be applied as a dynamic method (with certain limitations because of the narrowness of the tube) as well as a static method.

FIGURE 2. STIEPEL FOAM METER

Figure 3 represents the apparatus used by Arbuzov and Grebenshchikov (*2*) to measure the foam stability of saponin solutions. Ten cubic centimeters of the liquid are put in the buret, D, and go out at an even rate, one drop at a time, onto the porous filter. The container, A, is connected to a water pump, a mercury manometer, and an outlet valve, E. When the solution passes through the filtering medium it takes up air from the pores and a foam is produced in the cylinder. As soon as all the solution has thus been converted into foam, valve E is opened and the drainage of the foam is allowed to proceed under atmospheric pressure. The rate at which the liquid drains from the foam is measured, using the narrow graduated tube at the bottom of the cylinder. The apparatus is kept in a thermostat during the whole process.

It is considered undesirable by these authors to observe the breakdown of a foam while the foam remains under the influence of the conditions which caused its formation. This is really an expression of preference for a static rather than for a dynamic method. More significant is the attention that they draw to the fact that, by their method, the surface-active substance is equally distributed through the whole mass of foam, because liquid is turned into foam one drop at a time until no more liquid remains. In other methods, where the entire remaining body of the liquid is present while

the foam is being formed, the liquid is continually depleted of surface-active agent, so that succeeding portions of the foam are progressively different, yielding a nonuniform foam. Hence, the method of foam formation may influence the experimental value of foam stability. However, it would seem that this is important chiefly in very dilute solutions of surface-active agents.

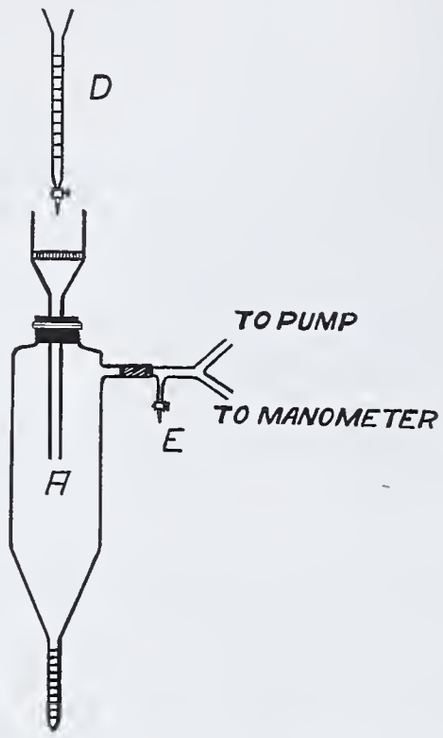

FIGURE 3. STATIC FOAM METER USED BY ARBUZOV AND GREBENSHCHIKOV (*2*, PAGE 33)

Arbuzov and Grebenshchikov (*2*) found that the exponential relation holds for the later half of the drainage of the foam, and they considered the reciprocal of the exponential constant to be a satisfactory unit of foam stability.

It would therefore appear that, with or without the conditions mentioned by Arbuzov and Grebenshchikov (*2*), the exponential relation is commonly obtained for drainage of a foam; moreover, results are reproducible within very wide experimental limits. There is little difficulty in correlating the results because of the same underlying relation for the drainage. In each case the total liquid collected is actually measured, and although collapse of the films takes place simultaneously, it is not measured as such but finds its place in the effect that a smaller volume of foam must exercise on the rate of drainage.

It is immediately recognized that where the foam possesses absolute stability, drainage of liquid is a purely hydrodynamic matter and has no relation at all to the foam stability. This has been pointed out by Ross and Miles (*14*), who suggest measuring the effect of an arbitrary standardized destructive mechanism acting upon a volume of foam during production under standard conditions and protected from adventitious destructive forces. This procedure is necessary only for foams of such stability that the films will drain without simultaneous or immediately subsequent collapse.

Dynamic Foam Methods

The lack of mathematical relations to express the opposing rates of growth and decay of a dynamic foam makes the task of correlating these methods considerably more complex than for static foam methods. In dynamic methods the emphasis is taken off the question of drainage from the foam and focused only upon the position of the topmost films. The effects that are actually measured are either the rate of rise of the foam in the measuring cylinder or the maximum constant volume of foam obtained, in both cases when a gas is passed continuously through a foam-producing liquid. It is, of course, impossible to prevent draining of the liquid from the foam and the consequent thinning and ultimate collapse of the liquid films, but only the rate of collapse is actually measured in dynamic methods. The rate of drainage, although it is not measured directly, nevertheless operates in the effect that a more rapid rate of drainage has in altering the rate of collapse of films.

This is in direct contrast to the static methods and the two together lead to the conclusion that the foaminess of a liquid, as it is usually measured, is a derived or subsidiary property, compounded of at least two quantities, drainage of films and collapse of films, themselves dependent on more fundamental properties such as viscosity, density, surface tension, vapor pressure, cohesion, and charge.

In most foams, whether measured by static or dynamic methods, it is difficult completely to segregate the drainage from the collapse of the films. Methods should be characterized by the relative emphasis given to these two quantities—that is, by the specific property measured rather than by the manner of measurement. This is more satisfactory than merely designating them as static or dynamic.

Bikerman Method

Bikerman (*3*) "aims at establishing foaminess as a definite physical property of a liquid, which must therefore be independent of the apparatus used and of the amount of material employed in measuring it". The apparatus he employed is a dynamic foam meter, of the kind recommended by Foulk and Miller (*6*) and by Lederer (*9*), as shown in Figure 4. Gas is forced at a measured rate through a porous membrane and an overlying layer of solution, as in most dynamic methods. The foam builds up until a constant maximum volume is reached.

The unit by which the foaminess is measured is designated as Σ and described as "the average lifetime of a bubble in the foam". The derivation of Σ throws some light on its character.

When dynamic equilibrium has been reached, the amount of foam maintains its constant maximum volume. At any time during this equilibrium the mass rate at which gas is being passed into the liquid is equal to the mass rate at which it is rising in the foam, and also to the mass rate at which it is escaping therefrom. In a tube of constant cross section linear rates can be used instead of mass rates. If the linear velocity of the gas in the foam is u when a volume of gas, V, is forced through the membrane in t seconds, then

$$u = V/tQ$$

where Q is the cross section of the measuring tube. If now, at equilibrium, the average maximum height of the foam layer is h, the expression h/u represents the time during which any arbitrary volume of gas is present in the foam. (The average maximum foam height is defined as the difference between the average maximum height attained by the liquid plus foam and the height of the liquid at rest.) It is this period of time that is Σ and the equation $\Sigma = h/u$ gives a precise definition—namely, the average lifetime of the gas in the foam. This might better be designated as L_g, because it is only in the rare case where there is no coalescence of bubbles within the foam that L_g becomes equal to the average lifetime of a bubble in the foam. It involves assumption and interpretation to take the next step, which is to call L_g the average lifetime of a bubble in the foam.

L_g is easily found experimentally. The quantities measured are the average maximum foam volume, v, and the volume of gas, V, passed through the membrane in time t. Then $L_g = h/u$, where $h = v/Q$ and $u = V/tQ$. Substitution leads to the expression $L_g = vt/V$.

The attainment of a constant volume of foam indicates equilibrium between building up of the foam by the in-going gas and the destruction of the top of the foam by the collapse of the topmost film or films. This is partly or often largely a matter of evaporation or contamination. With liquids of great foam-forming capacity the former process so far outweighs the latter that equilibrium, denoted by a constant maximum foam volume, is not practicable.

It can readily be seen that the actual average life of a bubble even in the foam cannot be defined by L_g unless there is no coalescence of bubbles before they disappear at the top of the foam. In actual practice, however, in many cases a single bubble present at the top of the foam may have started out as a large number of individual bubbles that have united somewhere on their upward journey to the top. Here L_g would not represent a true average lifetime of a bubble in the foam.

Smaller bubbles are more stable than larger bubbles. Hence Σ has a definite value for a given liquid only for a definite average size of bubble and a definite average amount of coalescence.

Another limitation on the meaning of Σ is introduced by its method of measurement. If, for illustration, a pure nonfoaming liquid is considered and air is passed through at a measured rate, there will be an increase in the volume of the sample due to the added volume of air present in it at any given time. This leads to a positive value of h and u and so provides a numerical value for the foaminess of a nonfoaming liquid. The error thus introduced is significant for poorly foaming or viscous liquids.

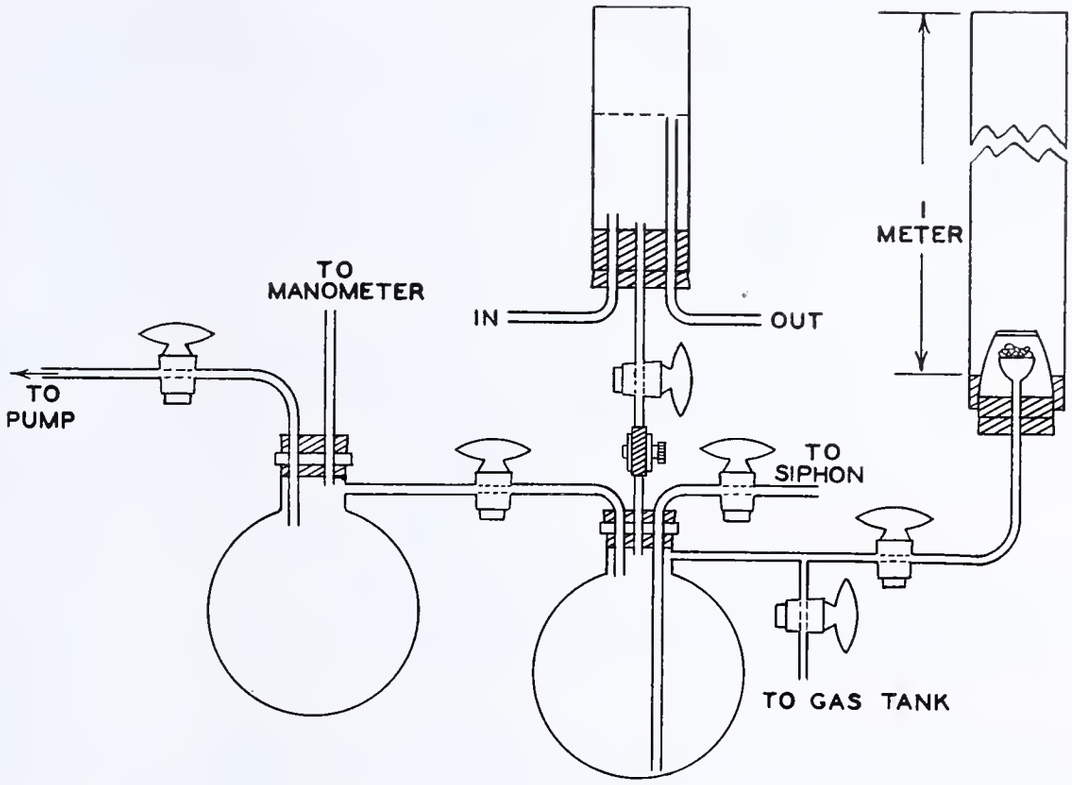

FIGURE 4. DYNAMIC FOAM METER

A paper by Hoffmann and Peter (*7A*) was not available until recently. Hoffmann and Peter used exactly the same unit as that originally proposed by Bikerman (*3*), though without any reference to his previous work. They used a capillary to produce a homogeneous foam of known bubble size. With hydrocarbon oils they observed only slight coalescence of bubbles in the body of the foam. For such foams it is permissible to interpret L_g as the average lifetime of a bubble, although the same reservations must be placed on that interpretation in the general case as have already been mentioned for the Bikerman unit. The linear rate of increase of the foam volume observed at the beginning of the foam production, although used by Mischke (*10*) and by Ostwald and Mischke (*11*) as a measurement of foaminess, is here more properly as a measurement of the rate of air input.

Effect of Size of Bubble

For a uniform film the chance of breaking is proportional to the exposed area, since breakage at one point causes collapse of the whole film; hence, the breakage of a bubble would depend upon the area exposed to the atmosphere or to other bubbles. Likewise, small bubbles are partly surrounded and protected by the mass of liquid in the Gibbs angle between them.

In general, therefore, the smaller a bubble the longer its life, which may be expected to be at least in inverse proportion to the square of the diameter of the bubble.

Conditions for Defining Rate of Collapse of Films

If the rate of collapse of film, or the foam stability, for one and the same liquid is to have a definite and significant numerical value, the following factors must be taken into account:

1. The size of the bubble, which should be stated.
2. The age of the solution.
3. The rate at which bubbles are formed.
4. The time taken for the determination, because if the time is protracted the rate of film collapse becomes abnormally slowed on account of the accumulation of stabilizing material on the remaining films.
5. In some cases carbon dioxide or reactive gases should be excluded from the atmosphere which is in contact with the foam. Carbon dioxide has a pronounced effect on stability of foam prepared from soap solutions and this perhaps may be true of some other surface-active agents.

It has been previously shown (*17*) that in addition to Nos. 2 and 3 there are two further factors which in practice can be important in affecting the stability of exposed film in an insufficiently enclosed apparatus. These are:

6. Temperature of the gas above the foam.
7. Partial vapor pressure above the foam for appreciably volatile solvents or solutes, often determined by the distance from the top of the foam to the top of the containing vessel.

Ostwald and Mischke Method

Ostwald, Siehr, and Mischke (*10–12*) have developed a dynamic foam meter in which gas bubbles of known size are passed into the liquid. For a constant flow of gas, the initial rate of rise of the foam in the measuring cylinder is taken as a measure of the foaming capacity of the liquid. In experiments with gelatin solutions between 10 and 0.1 per cent concentration Mischke found that the initial rate of rise is the same for all the samples and is constant for the first 3 minutes of foaming. This strongly suggests the possibility that it was characteristic of nothing more than the rate at which air was being passed into the liquid, in

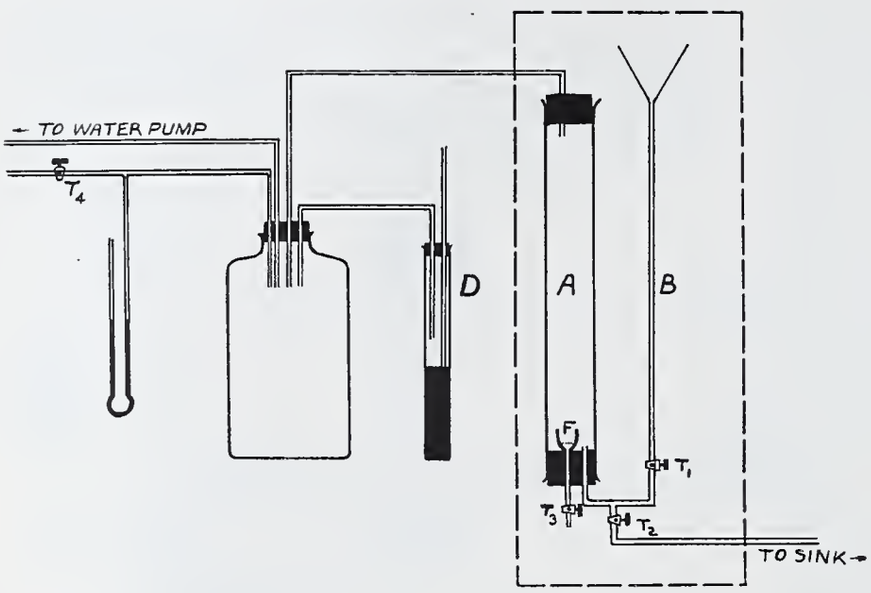

FIGURE 5. DYNAMIC FOAM METER USED BY PANKHURST (*13*, PAGE 497)

accordance with the idea of Lederer (*8, 9*) that the volume of foam produced from a strongly foaming solution is at first equal to the volume of air passed in. Experiments in this laboratory with dilute solutions of Aerosols have also proved the validity of this suggestion. It presents a formidable objection to their method, which is seen to be inapplicable to strongly foaming solutions.

The rate of formation of bubbles depends solely on the rate at which gas is bubbled through the liquid, itself determined by the difference in pressure, diameter of inlet tubes or pore diameters, viscosity of the gas and liquid, etc. Subtracted from this arbitrarily determined rate is the rate of collapse of films, the resulting rate being the experimentally determined quantity. The rate of film collapse cannot be a constant quantity, as not only must it depend on the amount of foam present, which is increasing steadily with the time, but also on propinquity (an isolated submerged bubble cannot break, even if infinitely unstable), and drainage, which is a nonlinear function of time (*15*). (Film collapse may indeed appear to be a linear function of time at the outset. Many complex functions can be approximated by a straight line when near the origin.) Consequently, if both kinetic factors are in simultaneous operation the experimentally determined rate of foam rise would not be constant, except possibly for a short time near the origin. It could be constant for a longer range only when film collapse is so delayed that it does not take place to any appreciable extent while the foam is being formed.

Pankhurst Method

Pankhurst (*13*) adopted a dynamic foam meter, a modification of that made by Foulk and Miller (*6*). A diagrammatic scheme of the apparatus is given in Figure 5.

The liquid under test is admitted to the large Pyrex tube, *A*, through the side tube, *B*. Tap T_1 is then closed and the level of the liquid in *A* is adjusted to an arbitrarily chosen zero mark above the porous septum, *F*, by means of tap T_2. Taps T_3 and T_4 are then closed and the pressure inside *A* is reduced to 10 cm. of mercury below that of the atmosphere and maintained by a constant-pressure device, *D*. When a steady pressure difference is obtained, air is admitted to the liquid in *A* through the porous septum, *F*, by opening T_3. The rate of intake of air is 1200 cc. per minute (±15 cc.). The time required for the advancing foam front to rise to a height of 50 cm. is measured with a stop watch. This value, θ_{50} seconds, is taken as an inverse measure of the foaming power of the liquid.

This method is similar in principle to that of Ostwald and Mischke and the objections to that method are also applicable to this one. It can be calculated from the information given about the apparatus that the air itself passing into tube *A* would require 99 seconds to rise 50 cm. in the tube. (Because of this fact one would not expect to find any values of θ_{50} less than 99 seconds, as it is hard to image any mechanism whereby the foam can rise at a faster rate than the rate at which the air is being passed into the liquid. Nevertheless, out of the 37 reported measurements of θ_{50} 21 have values less than 99 seconds.) Consequently, values of θ_{50} in the neighborhood of 99 seconds could not be taken as characteristic of the foaminess of the liquid under test.

Schütz Method

The gas is introduced by means of a single capillary, ending underneath the surface of the liquid (*17*). The amount of gas bubbled through the liquid is the same in every case, and corresponds to a reduction of pressure of the gas from 18.80 to 15.40 cm. of mercury. The apparatus is shown schematically in Figure 6.

E is a device for obtaining the same initial pressure of the gas and *J* is a sintered-glass filter to retain fine dust particles. The tube, *G*, has at its end a "thermometer capillary" ground to the shape shown in the inset of Figure 6. The period between the stoppage of the gas entry and the time when half the foam-covered surface of vessel *F* is free from bubbles, was called the "foam time", T_f, and used as a measure of foaminess. It is perhaps an unfortunate choice to use dissimilar quantities to define the extremities of the time period. To determine when exactly half the foam surface is gone, a wire circle of calculated

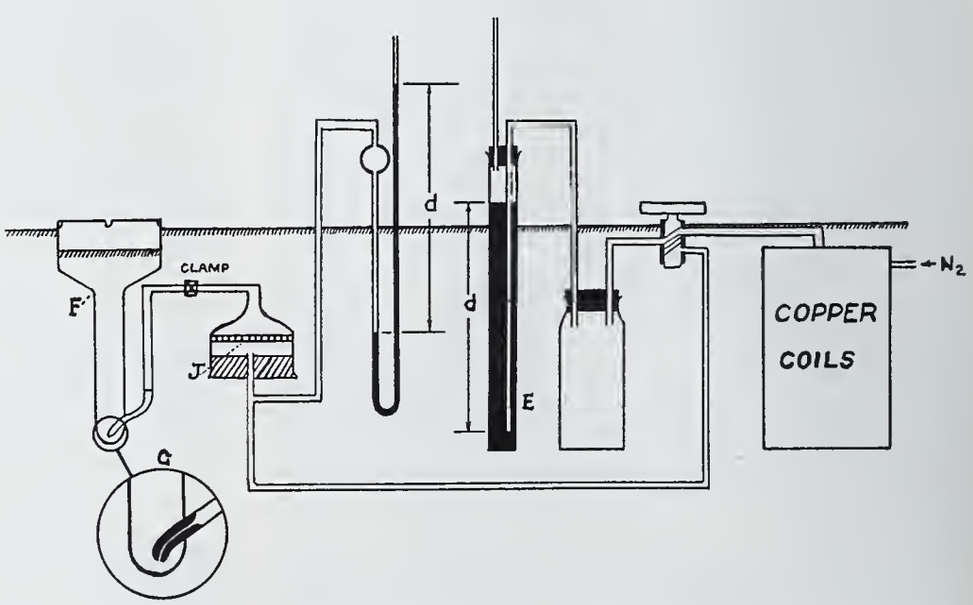

FIGURE 6. APPARATUS USED BY SCHÜTZ TO MEASURE FOAM TIME (*17*, PAGE 89)

diameter is placed on the edge of the vessel, at a fixed distance from the eye of the observer (Figure 7). In most cases the bubbles break first in the center, leaving the remainder grouped around the wall, so that observation of the half area, as shown in Figure 7, offers no difficulty.

Although the measurement is made after the foam has formed, yet it is the time taken for collapse of films that is actually measured, and hence this method must be grouped along with those that emphasize a function of collapse rate, as do most methods that employ a dynamic foam meter.

In this method unusual care is taken to make allowance for the influence of the variables that have previously been noted to affect the stability of films. The solutions were always of equal age, the temperature was maintained constant by a water bath at 21.4° C. (±0.2°), and the distance from the surface of the liquid to the upper edge of the vessel (a in Figure 7) was kept at a low value (2.5 cm.), it having been shown that with higher values of a the results were not reproducible.

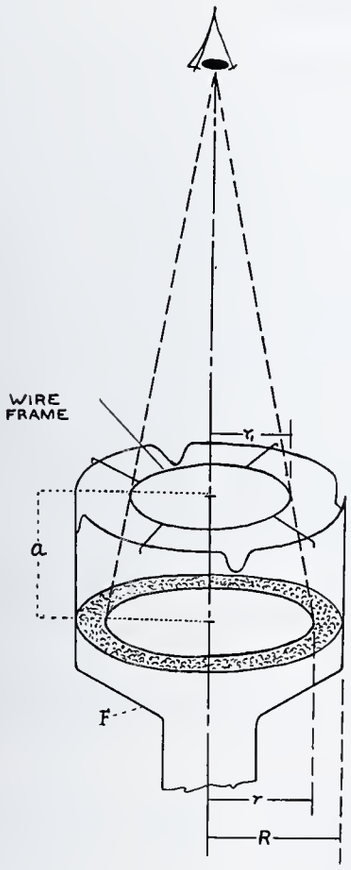

Figure 7. Device Used by Schütz to Observe When Half-Surface Area Is Clear of Foam (*17*, Page 87)

As with many of the methods previously discussed, this one too was inapplicable to strongly foaming solutions. For solutions of saponin greater than 0.001 per cent the values of T_f exceeded 50 minutes and, because of the difficulties of keeping conditions constant for so long a period of time, reproducible results could not be obtained. This particular method, however, would appear to be abnormal in this respect, as very much higher concentrations of saponin have previously been measured by other methods without undue difficulty (*2*, *13*).

Two improvements on the Schütz method are suggested. One of them is to restrict the amount of foam to a single layer of bubbles on the surface, in order to dispose of the effect of drainage as much as possible; and to take this as zero time. The second improvement is the result of the observation that bubbles break first in the center of the liquid surface only when there is a wall around the periphery. This variable, due to a wall effect, can be eliminated by having the liquid surface flush with the top of the container, when it is observed that the bubbles will break first at the edges and cluster around the center. The author has found this to be a general phenomenon, true for both aqueous and nonaqueous systems.

Results obtained by the Schütz method confirm the results of Arbuzov and Grebenshchikov (*2*) for the foaminess of saponin solutions as a function of concentration. The Russian investigators, using a concentration range of 0.2 to 2.0 per cent, found that the foaminess, measured by a drainage method, could be related to the concentration up to 1 per cent by an expression similar to the classical adsorption isotherm,

$$F = aC^b$$

where F is the foaminess, C is the concentration. and a and b are constants. At concentrations greater than 1 per cent the foaminess becomes practically constant. Schütz established the same mathematical relation for saponin solutions in the concentration range 10^{-3} to 10^{-5} per cent.

Single Bubble Methods

The relative simplicity of technique required for the production and observation of single isolated bubbles floating on a liquid surface, as well as the fact that a single bubble is more amenable to a theoretical mathematical treatment than is a bulk foam, makes it likely that single bubble methods are due to receive a greater prominence than heretofore. Such a method has already been put to good use, as, for instance, by Hardy (*7*) and by Talmud and Suchowolskaja (*18*), to gain information about the nature of interfaces. There is no doubt that reproducible results can be obtained, which have been of value in research.

It is known, and can readily be demonstrated, that several bubbles together are more stable than each separately, possibly on account of the entrained liquid in the rounded corners and edges between them, discussed by Gibbs. Nevertheless, experimental results obtained by C. G. Lindquist and the author in this laboratory indicate a correlation between the results of single bubble observations and some foam-measuring methods for certain nonaqueous systems. A difference in the order of magnitude of the numerical result is to be expected but the same sequence of stabilities for a series of different samples can be obtained. A more extensive report of those experiments cannot be given at the present time.

Acknowledgment

Thanks are due to James W. McBain for many helpful discussions.

Summary

1. The chief methods, static and dynamic, currently employed to measure foaminess of liquids have been examined to ascertain the factors involved, since each measures a somewhat different complex of properties, such as drainage, apart from mere collapse or coalescence of bubbles.

2. For a film of a given stability the average life of a bubble is greater the smaller the bubble, increasing at least in inverse proportion to the square of the diameter of the bubble

3. Methods have been suggested for examining the stability of foams with a minimum of complications through other factors.

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Presented before the Division of Colloid Chemistry at the 104th Meeting of the American Chemical Society, Buffalo, N. Y.

Baumé–Dry Substance Tables for Starch Suspensions

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No tables for Baumé–dry substance starch have been published in this country, although it is a large producer of starch. Such tables are presented for cornstarch, together with a new method for moisture on starch suspensions. The experimental evidence indicates that such tables can be constructed mathematically if the absolute density of the specific starch is known.

FOR many years the wet-milling industry in this country has employed the hydrometer for measuring the density of starch suspensions or slurries in their manufacturing operations. Various companies have adopted dry substance tables for these Baumé values, but no common table exists for use within or without the industry where starch is employed.

The use of a hydrometer for the measurement of solids in a suspension or slurry may seem strange to chemists who regard it solely from the standpoint of determination of density in true solutions. Its success in starch suspensions is based on the relatively slow settling of the starch and on the fact that the concentration of the starch suspensions at the hydrometer bulb is substantially constant. An analyst familiar with these properties acquires the technique readily and results on a common sample agree within 0.1 Bé. or 0.2 per cent dry substance.

Tables have been published for the Baumé–dry substance relationship for potato starch suspensions (*4*, *5*, *6*). Other papers have been published on the densities of the various starches (*3*).

The purpose of this paper is to present such tables for cornstarch, known in the trade as pearl starch, for the hydrometer in use in this country—145 Modulus, standardized at 60° F.

Experimental

This work is based on methods previously published on the determination of the Baumé–dextrose equivalent–dry substance for corn sirup and corn sugar (*1*, *2*) which should be consulted for the basic experimental methods, as only the departures from this work are discussed here.

Cornstarch. The starch used for these tables was taken from the final washing filter in the factory. The cake from this filter is either dried to form the pearl starch of commerce or suspended in softened water for subsequent hydrolysis to corn sirup or corn sugar. A quantity of this suspension was washed by decantation or dewatered on a Büchner funnel, washed, and finally suspended in distilled water to approximate the various densities required. The starch had a crude protein content of 0.3 per cent on a dry basis.

Baumé by Hydrometer. The Baumé readings were made at three temperatures—60°, 100°, and 140° F. (15.56°, 37.78°, and 60° C.), the temperature of the bath being maintained within ±0.02° F. (0.011° C.). Approximately a gallon (3.785 liters) of the sample was placed in a glass bottle in the water bath and the suspension was maintained by a motor-driven stirrer so adjusted that the agitation was not vigorous enough to cause air to be occluded. When the temperature of the suspension equalled that of the bath, the sample was quickly transferred to hydrom-

Table I. Baumé–Per Cent Dry Substance Starch

Baumé	Corresponding Specific Gravity (Air)	F = 1.7770 60°/60° F.	F = 1.7700 100°/60° F.	F = 1.753 140°/60° F.
−2.01	0.9864	...	...	0.00
−0.71	0.9951	...	0.00	2.28
0.00	1.0000	0.00	1.26	3.52
1.00	1.0069	1.78	3.03	5.28
2.00	1.0104	3.55	4.80	7.02
3.00	1.0211	5.33	6.57	8.78
4.00	1.0285	7.11	8.34	10.54
5.00	1.0358	8.89	10.11	12.29
6.00	1.0433	10.66	11.87	14.04
7.00	1.0568	12.44	13.65	15.79
8.00	1.0585	14.22	15.42	17.55
9.00	1.0663	15.99	17.19	19.30
10.00	1.0742	17.77	18.96	21.05
11.00	1.0822	19.55	20.73	22.81
12.00	1.0903	21.32	22.50	24.56
13.00	1.0986	23.10	24.27	26.31
14.00	1.1071	24.88	26.04	28.08
15.00	1.1156	26.66	27.81	29.82
16.00	1.1242	28.43	29.58	31.57
17.00	1.1330	30.21	31.35	33.32
18.00	1.1419	31.99	33.12	35.08
19.00	1.1510	33.76	34.89	36.83
20.00	1.1602	35.54	36.66	38.58
21.00	1.1696	37.32	38.43	40.34
22.00	1.1791	39.09	40.20	42.09
23.00	1.1888	40.87	41.97	43.84
24.00	1.1986	42.65	43.74	45.60
25.00	1.2086	44.43	45.51	47.35

[1] Present address, Goodyear Tire & Rubber Co., Akron, Ohio.

Table II. Corrections to Be Subtracted from Assigned Specific Gravity to Give True Specific Gravity in Vacuum

Baumé	Assigned Specific Gravity (Air)	Corrections to Be Subtracted 60°/60° F.	100°/60° F.	140°/60° F.
−2.01	0.9864	...	...	0.00206
−0.71	0.9951	...	0.00105	0.00213
0.00	1.0000	0.0000	0.00111	0.00216
5.00	1.0358	0.00005	0.00120	0.00233
10.00	1.0742	0.00012	0.00130	0.00233
15.00	1.1156	0.00019	0.00143	0.00265
20.00	1.1602	0.00025	0.00154	0.00281
25.00	1.2086	0.00031	0.00165	0.00298

Table III. Temperature Corrections to Be Added

Baumé	100° to 60° F.	140° to 60° F.	120° to 60° F.	120° to 100° F.
0.00	0.71	1.98	1.24	0.52
5.00	0.69	1.92	1.20	0.51
10.00	0.67	1.85	1.17	0.49
15.00	0.65	1.78	1.13	0.48
20.00	0.63	1.72	1.09	0.46
25.00	0.61	1.65	1.05	0.44

eter cylinders and five 4-ounce (0.12-liter) screw-top bottles. The former were used for the determination of Baumé by hydrometer and the latter for determination of density by pycnometer.

The starch in the cylinders was kept in suspension by means of a long rod which had a perforated disk at one end, and by carefully introducing and manipulating this agitator, no air was carried into the suspension. The hydrometer to be used was kept in a cylinder of distilled water adjacent to the cylinders containing the starch suspension, and when the temperature of the starch suspension was the same as that of the bath, the hydrometer was removed, quickly wiped free from the film of water, and placed in the starch suspension. When the spindle was at rest a small drop of methylene blue solution (in water) was added at the stem to accentuate the meniscus and the reading made at the upper edge of the blue line around the stem. Previous work had indicated that the differences between the reading of this point and the plane surface of the liquid (correct reading) was 0.08° Bé. Thus 0.08° Bé. was added to the observed reading and this value appears in all tabulated data for Baumé.

Density by Pycnometer. Settling difficulties presented a problem in the determinations of density by pycnometer and as a result three methods were used to overcome this trouble. None is completely free from criticism, although the latter two are regarded as more desirable. The pycnometers used have been described previously (*2*).

a. The 4-ounce samples mentioned above were kept in the bath while the Baumé was determined by hydrometer. Then the temperature was raised or lowered a few degrees, depending on relationship of bath to room temperature, to compensate for heat changes during the transfer of the sample to the pycnometer. The bottle was agitated every 5 minutes, then returned to the bath, reagitated, etc. After five such agitations, the sample was transferred rapidly to the pycnometer, stoppered, and placed in the bath which was now set at the desired temperature. The pycnometers were kept in the bath for 30 minutes, then removed and weighed.

Using this method, difficulty was encountered at the higher concentrations in that starch collected at the ground surfaces. The precision was about 0.05° Bé. and as a result this method was discarded for the heavier Baumés and method *b* used instead.

The moisture on the starch was determined on one of the remaining 4-ounce samples, as described below.

b. The pycnometers were filled about three-fourths full at room temperature, heated to about 130° F., and boiled very gently under high vacuum to remove occluded gas. The starch was then allowed to settle and the remaining space was filled with cool, recently boiled distilled water. The pycnometer was placed in the water bath and weighed at the end of an hour. From this weight it was possible to calculate the specific gravity on the basis of complete suspension.

This method eliminated the difficulty of the collection of starch between seals and also enabled any loss through the capillary of the stoppers to be taken as pure water—i. e., no solids. The moisture test was made on a separate bottle as before.

c. The pycnometer was used with an added feature consisting of a test tube with standard-taper joint as shown in Figure 1. The starch suspension was run into the pycnometer until it was nearly full and then allowed to settle. When the top part of the liquid was essentially clear, the stopper was inserted and the apparatus placed in the bath. After an hour the pycnometer was removed from the bath and weighed. Then the stopper and outer cap were removed and the test tube was applied. The suspension was shaken until uniform and a moisture determination made on this material.

This method eliminated the difficulties of starch at the seals; the liquid that was lost through the capillary was essentially pure water; and settling was not a dominant factor, since the moisture determination was made on the suspension actually present in the pycnometer. Tests were made to estimate the error introduced by loss of the film of water left on the stopper and cap which were removed when the test tube was attached for resuspending the starch. This was found to be 20 to 30 mg. on a 95- to 100-gram sample weight. This involves an error of 0.02 to 0.03 per cent and can be neglected for all ordinary purposes or be compensated for in work of highest precision.

Determination of Moisture. The method used is basically the same as that employed for the determination of moisture in corn sirup and corn sugar (*1*) which should be consulted for details.

Diatomaceous Silica, prepared Johns-Manville Hy-Flo (*1*, *2*).

Apparatus. Wide-mouth Erlenmeyer flasks, 250-ml. capacity, with 40/12 standard taper, with stoppers. Pyrex test tubes 100 × 15 mm. (*1*).

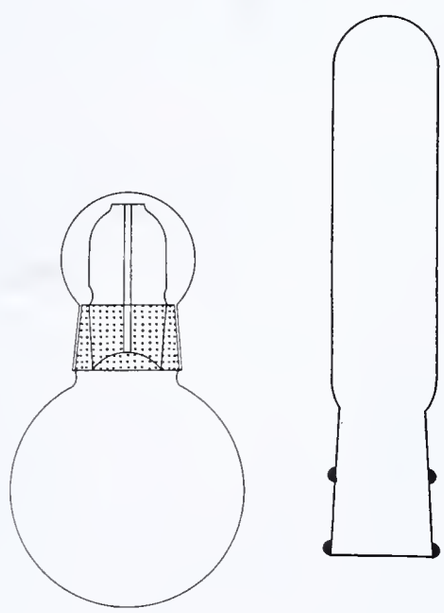

Figure 1

Procedure. Twenty-five to 35 grams of diatomaceous silica were run into duplicate flasks, the test tube was introduced, and weight constancy was obtained under oven conditions identical with those of the test. Weighings were made with an empty flask as a tare. The starch samples, either in the 4-ounce bottles or the pycnometer equipped with test tube, were shaken until homogeneous suspensions were obtained. Sample portions, sufficiently large to yield 5 to 8 grams of dry substance, were transferred quickly to the flasks by means of a pipet from which the tip had been cut. The sample was then worked into the diatomaceous silica by means of the test tube, which yielded a damp powdery mass.

The flasks were placed in a vacuum oven at 100° C., and the pressure was reduced first by an efficient water pump until most of the water was removed and then by means of a Megavac pump. After approximately 4 hours of drying the flasks were removed

Table IV. Baumé–Dry Substance Starch

(100°/60° F., F = 1.7700, 10° hydrometer)

Baumé	Specific Gravity (Air)	Dry Substance	Pounds per Gallon	Pounds Dry Substance per Gallon
0.00	1.0000	1.26	8.328	0.105
1.00	1.0069	3.03	8.386	0.254
2.00	1.0140	4.80	8.445	0.405
3.00	1.0211	6.57	8.504	0.559
4.00	1.0285	8.34	8.565	0.714
5.00	1.0358	10.11	8.626	0.872
6.00	1.0433	11.88	8.689	1.032
7.00	1.0508	13.65	8.751	1.195
8.00	1.0585	15.42	8.815	1.359
9.00	1.0663	17.19	8.880	1.526
10.00	1.0742	18.96	8.946	1.696
11.00	1.0822	20.72	9.013	1.867
12.00	1.0903	22.50	9.081	2.043
13.00	1.0986	24.27	9.150	2.221
14.00	1.1071	26.04	9.220	2.401
15.00	1.1156	27.81	9.291	2.584
16.00	1.1242	29.58	9.362	2.769
17.00	1.1330	31.34	9.436	2.957
18.00	1.1419	33.11	9.510	3.148
19.00	1.1510	34.88	9.586	3.344
20.00	1.1602	36.65	9.662	3.541
21.00	1.1696	38.42	9.741	3.742
22.00	1.1791	40.19	9.820	3.947
23.00	1.1888	41.96	9.900	4.154
24.00	1.1986	43.73	9.982	4.365
25.00	1.2086	45.50	10.065	4.580

from the oven and cooled and the mass in the flasks was reworked into a fine powder. The flasks were returned to the oven, and a pressure of less than 1 mm. maintained until weight constancy was obtained. An overnight period has been found adequate. Because of the very hygroscopic property of dry starch, an efficient train (*1*) must be used to introduce air into the oven and the flask stoppers must be inserted immediately upon opening the oven.

This method gave reproducible results within very narrow tolerance. Microscopic examination of the dried starch revealed no visible change in the granules through rupture or gelatinization. Tests indicated that oven temperatures could be carried as high as 110° C. with no appreciable effect on the results.

The method of reporting data was the same as that previously used (*2*). The net pycnometer weight was corrected first to vacuum. In the case of the 60°/60° F. data, the density (vacuum) was obtained and the corresponding Baumé value assigned. This procedure was changed for 140°/60° F. and 100°/60° F. data. After the net pycnometer weights had been corrected to vacuum, an additional weight correction (glass expansion and hydrometer) was added before calculating the density in order to make these values agree with the corresponding hydrometer readings. The reason for this has been previously discussed (*2*). By this correction observed Baumé readings and Baumé values obtained by pycnometer always agreed within 0.05° Bé. and with an average deviation of 0.03° Bé.

The Baumé–dry substance data were plotted in two ways: Baumé *vs.* dry substance and Baumé *vs.* factors. In both cases the best curve was a straight line, as it should be if the slurry were a true suspension. The previously used method of factors was employed for purposes of tabulation.

The factors were:

$$\text{Factor for } 60°/60° \text{ F. } (F60°/60° \text{ F.}) = \frac{D.S.}{\text{Bé.}} = 1.7770$$

$$\text{Factor for } 100°/60° \text{ F. } (F100°/60° \text{ F.}) = \frac{D.S.}{\text{Bé.} + 0.71°} = 1.7700$$

$$\text{Factor for } 140°/60° \text{ F. } (F140°/60° \text{ F.}) = \frac{D.S.}{\text{Bé.} + 2.01°} = 1.753$$

In Table I, the Baumés in all cases are the observed values and the specific gravities (air) are those corresponding to the Baumés.

In the above data the specific gravities at 60°/60° F. in air can be converted to vacuum by the usual calculations. The 100°/60° F. and the 140°/60° F. data require not only the calculation for air to vacuum but also those for glass expansion and for hydrometer. This has been discussed previously (*2*) and an abridged table (Table II) is given here for those who may desire to express the data on some other basis.

Since most Baumé tests in factory processes are not made at a fixed temperature, a table of temperature corrections is necessary (Table III).

The determination of each point in the table furnished enough data for calculation of the density of the actual starch solids. Hence a series of independent starch density values were obtained over the range of suspension gravities. These were observed to check very closely in the 60°/60° F. series and to average 1.636. Employing this value for the Baumé–dry substance relationship, a calculated table was found to check the experimental table within the limits of the experimental methods used. It would appear, therefore, that tables for water suspensions of the whole gravity range could be prepared for any starch from an accurate density value of starch solids determined at a single point. Each temperature, of course, would require a separate determination.

A table has been prepared for general factory use based on 100° F (Table IV). In this table the Baumés are the observed readings, the specific gravities are those in air assigned to the Baumé values and the weights per gallon are based on these specific gravities.

Briefly, the corrections (Table V) mean that when the 100°/60° F. table is used: for each 5° F. below 100° F., subtract 0.1° Bé. from the reading; for each 4° F. above 100° F., add 0.1° Bé. to the reading.

Table V. Temperature Corrections in Baumé

(To be added to or subtracted from observed Baumé to adjust to 100° F.)

Temperature, ° F.	Observed Baumé (10° Hydrometer) 0	5	10	15	20	25
	Subtracted from Observed Baumé					
80	0.40	0.39	0.38	0.37	0.36	0.35
85	0.31	0.31	0.29	0.29	0.28	0.27
90	0.21	0.21	0.20	0.20	0.19	0.19
95	0.11	0.11	0.11	0.10	0.10	0.10
	Added to Observed Baumé					
104	0.09	0.09	0.09	0.08	0.08	0.08
108	0.19	0.19	0.18	0.17	0.17	0.16
112	0.30	0.28	0.28	0.27	0.26	0.25
116	0.41	0.39	0.38	0.37	0.36	0.34
120	0.52	0.51	0.49	0.48	0.46	0.44

Conclusions

Experimental results indicate that tables for Baumé–dry substance starch can be made mathematically, provided the density of any starch is known.

A procedure for drying starch slurries or suspensions is presented.

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Dual Alternating Current Titrometer

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An instrument is described which comprises two complete titration stands and an alternating current-operated electrometer which allows the use of any electrode system in aqueous or nonaqueous solutions. Meter scales and potentiometer circuits are provided which give the instrument a range of −1.65 to +1.65 volts, readable to 0.5 millivolt, and with a calomel-glass electrode system, a full pH range readable to 0.02 pH unit. The stability is excellent and the grid current of the order of 10^{-12} ampere.

THE increased use of sturdy glass electrodes and nonaqueous, high-resistance titration media in potentiometric titrations has emphasized the need for a versatile potentiometer, or millivoltmeter (*5*), that will satisfactorily measure the potential between electrode terminals of a cell having a resistance up to 5000 megohms. The final step in the development of an ideal laboratory titrometer—namely, the elimination of batteries with their attendant replacement problem and constant drift—is the subject of the present paper.

The dual alternating current titrometer, like the battery-operated instrument previously described, is continuously indicating and employs a step potentiometer in combination with the vacuum tube electrometer. The use of a continuous indicating meter not only requires less manipulation by the operator, but also enables him easily and definitely to ascertain when the potential reaches equilibrium. The step potentiometer makes it possible to meet the requirement of wide range along with high sensitivity in a single indicating meter.

Figure 1 is a photograph of the instrument showing the housing which contains the electrometer circuit and supports the titration stands.

Accessory Equipment

The convenient titration stand previously described (*3*) has been incorporated to provide a complete titration unit which can be used for routine as well as special determinations.

Electrical Circuit

The electrometer circuit, shown in Figure 2, is of the single-stage, balanced-input type (*2*, *7*). Careful circuit balance and large values of negative feedback have resulted in a highly stable circuit which has negligible zero-drift when operated under moderate conditions of line voltage variation.

The input grid current is of the order of 10^{-12} ampere. A minimum value of grid current is obtained by varying the plate voltage of the electrometer tubes so that the grid voltage–grid current curve is shifted along the voltage axis until zero grid current is obtained for an input voltage of plus 100 to 150 millivolts. When this condition is fulfilled the maximum grid current for both positive and negative input voltages is approximately equal and occurs at full-scale deflection. A typical E_g–I_g curve for an average tube is shown in Figure 3; the grid current does not exceed 3.7×10^{-12} ampere in any part of the range from −250 to +250 millivolts.

The calibration of the linear scale is within the 2 per cent error of the meter used. Individual calibration of the scale would materially increase the accuracy of the instrument.

Two Type 38 tubes are used as the electrometer tubes. The rectifier is a type 6ZY5G, and a VR105 serves as a voltage regulator. Total power consumption is approximately 10 watts. The vacuum tubes may be expected to have long life with this instrument because they are all operated well below their normal ratings. The replacement of the rectifier tube (6ZY5G) should require no circuit adjustments. Replacement of the voltage regulator (VR105) may require an adjustment of the potentiometer circuit.

Controls

There are six panel controls: three switches and three variable resistors. The switches, requiring more frequent manipulation than the resistors, are placed at the bottom of the sloping front panel where they are convenient to reach. The 12-point switch,

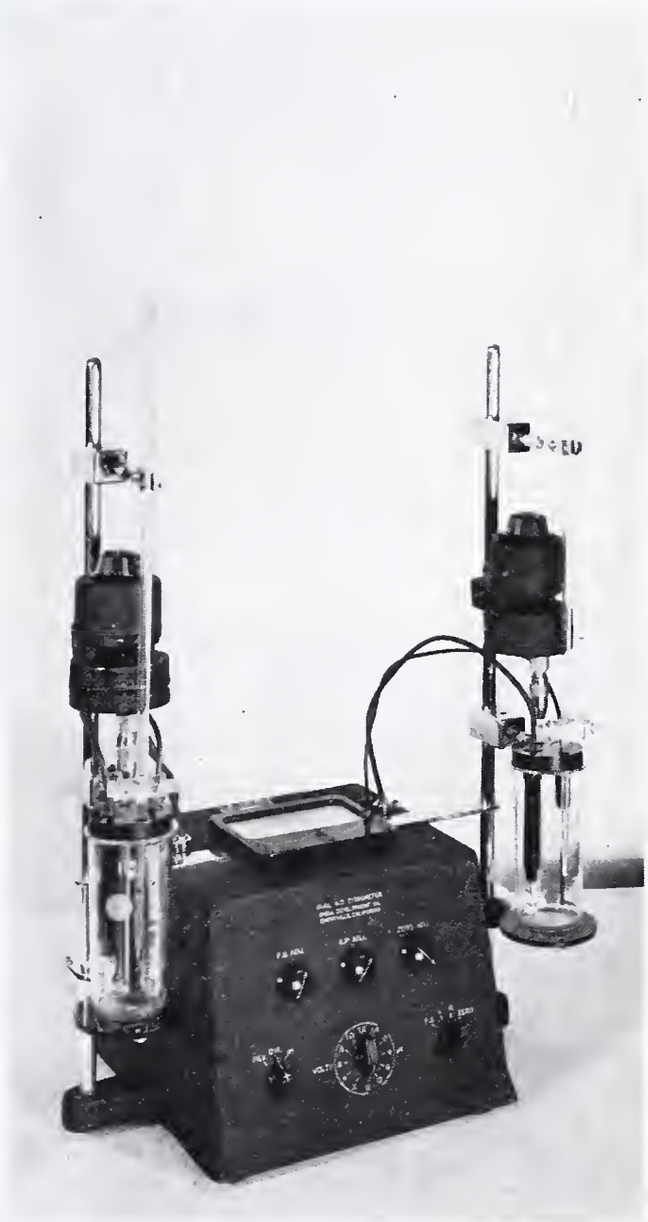

FIGURE 1. DUAL ALTERNATING CURRENT TITROMETER

S_3, controls the potentiometer circuits for both the millivolt and pH ranges. Switch S_2 reverses the meter and also sets up the circuit for the pH calibration. Switch S_1 shorts the electrode leads for zero adjustment, and connects them to a source of 250 millivolts for full-scale adjustment, and to either the right- or left-side electrodes for potential or pH measurements. The variable resistor controls are for zero and full-scale adjustment and for compensation of variations in asymmetry potential of individual glass electrodes.

In addition to the three panel controls, there are three controls mounted on the chassis inside the cabinet. One of these is a filament series resistor, *FIL.*, for tube balance, another aids in obtaining minimum grid current by adjusting the plate voltage, *P.V.*, and a third, *STD*, adjusts the main voltage divider to standard voltage.

Power Supply

All the direct current voltages are obtained from a voltage divider which is maintained at constant potential by the regulator tube. Plus or minus 10 volts' change in line voltage (115 volts) results in a change in direct current voltage of ±0.1 per cent.

No power switches are provided; the rheostats on the motors have "off" positions against the counterclockwise stop, and it is intended that the electrometer circuit be operating continuously where it is in steady or intermittent use. The permanent tem-

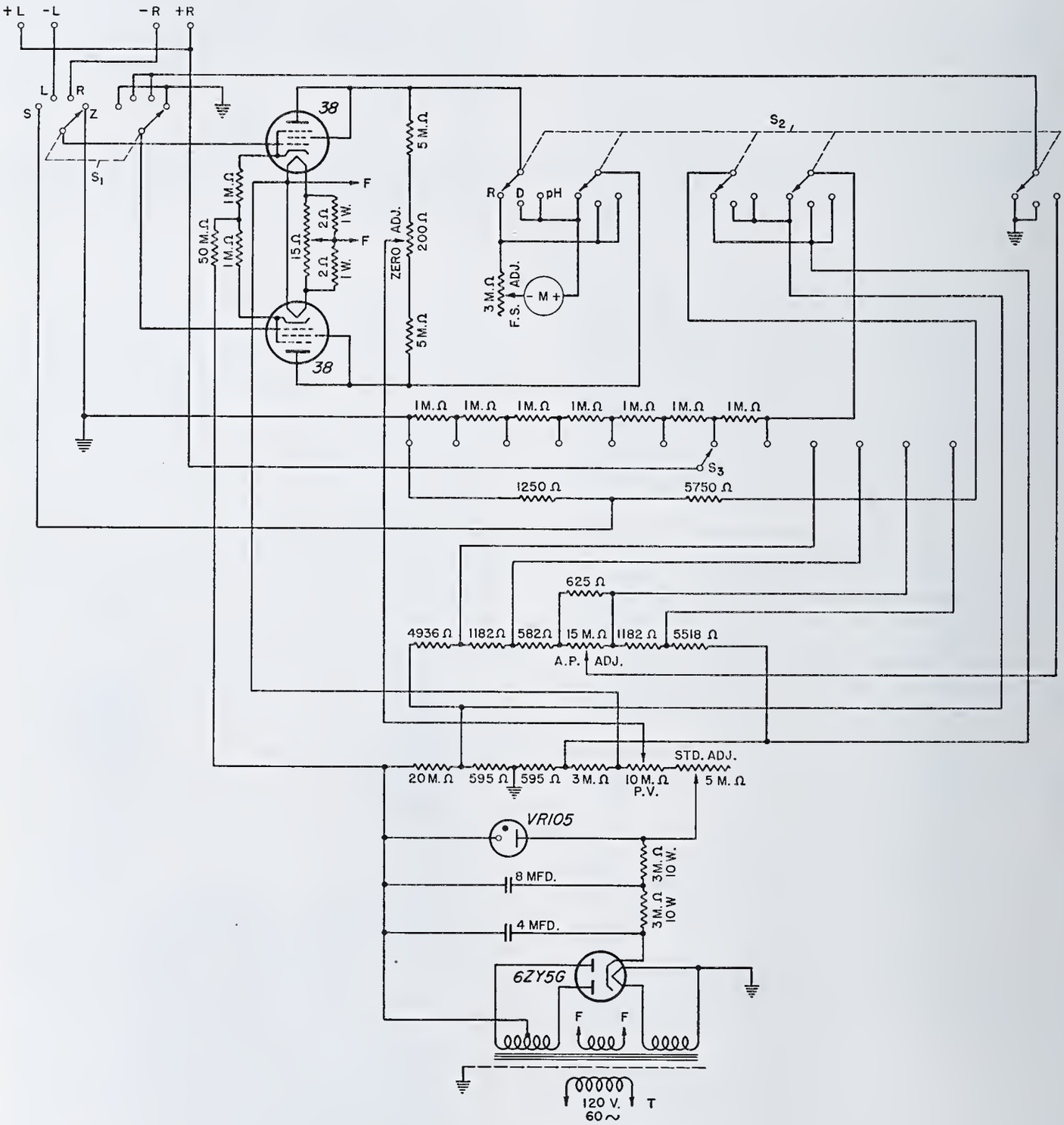

FIGURE 2. WIRING DIAGRAM OF TITROMETER

M. Simpson Model 29, 0- to 25-milliampere direct current meter
S₁. Centralab Isolantite switch 2505
S₂. Centralab Bakelite Switch 1417
S₃. Yaxley switch 32112J
T. Specialty Division, Gardner Electrical Mfg. Co., Emeryville, Calif., transformer S 3690
Variable resistors, Yaxley Type 4 MP
Fixed resistors, Type R, Precision Resistor Co. W. W. ±1 per cent unless otherwise noted

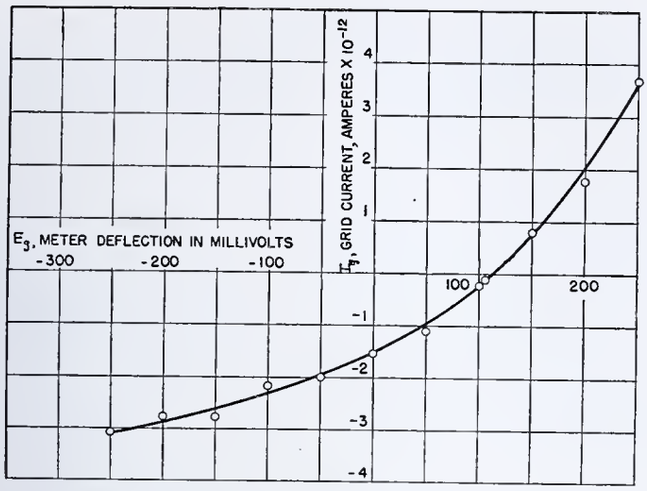

FIGURE 3. GRID CURRENT TYPE 38 TUBES

perature equilibrium thus established results in more stable operation, and the very low power consumption makes continuous operation entirely practical.

Constructional Details

In order that the instrument may function on a high input resistance, all connections to the "high" tube must be exceedingly well insulated. Switch S_1 should be Isolantite-insulated, the spring contact for the glass electrode connection should be supported on Isolantite or polystyrene, and the interconnecting leads should be of rigid bus-bar suspended in air between end terminals.

The envelope of the 38 electrometer tube is coated with ceresin wax to prevent surface leakage. The envelopes are first carefully cleaned and then immersed in the molten wax (approximately 140° C.) until all bubbling stops; the grid cap must be freed of wax before the tube is placed in service.

The instrument must be completely enclosed in a metal box, with the high input terminal located well within the box. A shielded-lead type of glass electrode is necessary (*1*). Sheet iron can be used for all the box except the meter panel, which must be of nonmagnetic material because of the shunting effect of steel on the meter.

Lubricating the wires and sliding contacts of all the variable controls with a good grease is necessary to prevent fluctuations caused by poor contact.

Circuit Adjustments

After the tubes are installed the instrument is plugged into the power outlet and allowed to reach temperature equilibrium. As the tubes warm up, the meter needle will swing across the dial once or twice and then approach a zero reading.

ZERO ADJUSTMENT. Switch S_1 is placed on *ZERO*, the panel knob, *ZERO ADJ.*, is set in the center of its travel, and the chassis-mounted filament rheostat, *FIL.*, is adjusted until the meter reads zero deflection. It may be necessary to interchange the tubes (Type 38) to complete this adjustment. The needle may be adjusted to an exact zero reading by means of the *ZERO ADJ.* knob. Further zero adjustments will be required at longer intervals until the instrument has reached a temperature equilibrium.

STANDARD ADJUSTMENT. One thousand millivolts from an accurate external potentiometer are applied to the input leads and switch S_3 is set at 1.0 volt. The meter should indicate zero deflection and may be adjusted to that value by means of the chassis-mounted control, *STD.*

FULL-SCALE ADJUSTMENT. Switch S_1 is placed on *F.S.* and the *F.S. ADJ.* rheostat varied until the meter indicates full scale.

MINIMUM GRID CURRENT ADJUSTMENT. (1) The glass electrode terminals are removed from their sockets.

2. Switch S_1 is placed on *R* or *L*, and switch S_2 on *REV.*, and the electrometer circuit is then completely shielded.

3. Potentiometer *P.V.* is adjusted so that the meter needle floats near mid-scale (100 to 150 mv.). This is done by means of a screw driver inserted through holes provided in the bottom plate of the cabinet. Since there is a slight interlocking of controls, it is advisable to check the full-scale adjustment. *F.S.*, after setting *P.V.*

If e. m. f. is to be measured, no other adjustments are required, but if a glass electrode system is to be used for pH readings, it will be necessary to make an adjustment for the asymmetry potential of the particular glass electrode used. To make this adjustment, switch S_2 is placed in the pH position, and if the usual pH 7 buffer is used, switch S_3 in placed on 4 pH. Switch S_1 is next placed on *L* if the left-side electrodes are used, or on *R* if the right-side electrodes are used, and the *A.P. ADJ.* knob is varied until the meter indicates 3 pH (a total reading of 7 pH—i. e., 4 pH on the potentiometer switch and 3 pH on the meter). Only one asymmetry potential adjustment is provided, since potential equilibrium is ordinarily reached so rapidly in aqueous solutions that there is no advantage in making pH determination alternately in two separate systems.

When potential measurements are made, the potentiometer switch is advanced from the zero position until the meter needle deflects on scale. The measured potential is then the sum of the potentiometer setting plus the meter reading.

If the meter indication is below zero when the potentiometer is on zero, the reversing switch, S_2, must be placed in its alternative position. When this switch is in the *D* (direct) position, the glass electrode is negative—that is, negative in the usual sense as applied to batteries and indicating electrical meters.

Operating Characteristics

Because of the electrical characteristics of the circuit, the meter may safely be used to measure any potential without previous knowledge of the magnitude or sign of the potential being measured, and without danger of polarization of the electrode system.

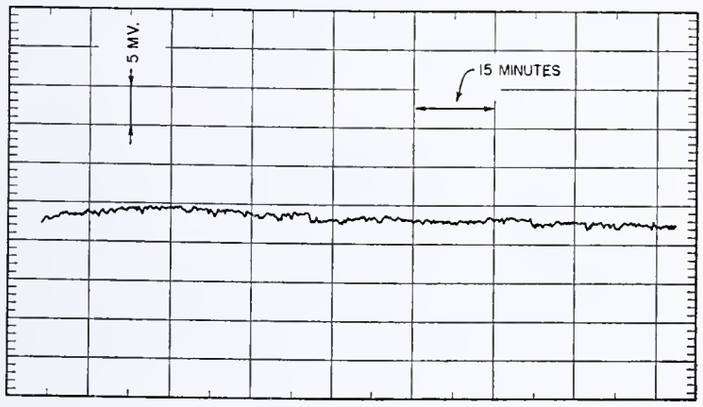

FIGURE 4. ZERO STABILITY RUN

Figure 4 is a record of a 2-hour stability run made without line voltage regulation. The maximum deviation over this period is less than ±1.5 millivolts and the momentary deviations are about ±0.5 millivolt. In locations having severe voltage fluctuations a transformer-type regulator will provide a perfectly stable zero.

Uses

In addition to all the usual applications involving electrometric titrations using high-resistance glass, silver, platinum, antimony, and tungsten electrodes with their corresponding reference electrodes, and for spot determinations of pH, the instrument has been particularly useful in mercaptan determinations (*4, 8, 9*).

The dual feature permits two titrations to progress simultaneously, which approximately doubles the work capacity for lengthy titrations in nonaqueous media, such as determination of neutralization number of lubricating oils (*1*). Alternatively two diverse electrode systems may be left permanently set up, so that, for example, both acidity and sulfur titrations may be made without delay on the single unit.

SPECIAL APPLICATIONS. The entire circuit has also been built into the case of a standard Model R, Leeds & Northrup,

recorder-controller. It has a range of 8 pH units, adjustable within the range 0 to 14 pH.

The circuit has also been successfully used for some time in a semiautomatic titrating device (*6*).

Acknowledgment

The authors wish to express their appreciation of the encouragement of L. Lykken and D. J. Pompeo.

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Colored Chromatograms with Higher Fatty Acids

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THE higher fatty acids, being colorless compounds of similar chemical and physical properties, present difficulties in their chromatographic separation, and such separation by several workers (*5*, *6*, *7*) has been successful only to a limited degree.

Cassidy (*1*, *2*, *3*) has made a careful study of the adsorption isotherms for various fatty acids in relation to their resolvability upon columns of various adsorbents. Among the obstacles encountered in using chromatographic columns has been inability to establish the location of the zones which define the separation of the various fatty acids in the mixture. This difficulty has been overcome in some colorless compounds (*10*, *11*, *13*), but none of these methods has been shown to be applicable in the case of fatty acids. Martin and Synge (*8*) applied the principle of producing a color change on an adsorbent to the chromatographic separation of the acetyl derivatives of the higher monoamino acids but they employed two liquid phases. Trappe (*12*) observed that silica-gel columns became transparent or translucent when wetted by certain solvents, and under these conditions it was possible to observe zones formed in the study of lipid mixtures.

This paper describes a method for separating mixtures of certain higher fatty acids into their components with the aid of adsorption columns, using an adsorbent impregnated with a dye to serve as an indicator. To be suitable for following the adsorption of fatty acids on an adsorbent, an indicator must change color on the column when in contact with the fatty acid, it must be insoluble in both the solvent for the fatty acid and the eluting agent and, in addition, it must revert to its original color after elution of the fatty acid. Preliminary experiments showed that phenolsulfonphthalein (phenol red) as manufactured by the Eastman Kodak Company satisfies the above conditions. Columns prepared with heavy magnesium oxide impregnated with this indicator proved satisfactory in bringing about and observing a separation. (Good results were obtained only when the heavy magnesium oxide manufactured by the J. T. Baker Chemical Company and the phenol red manufactured by the Eastman Kodak Company were used.)

The fatty acids were recovered from the adsorbent by dissolving the magnesium oxide in particular sections of the column (*4*) in concentrated hydrochloric acid and water, and subsequently extracting the fatty acid with diethyl ether. Magnesium oxide was found to be particularly adaptable to this procedure, in that it is readily soluble in the acidified water.

Reagents

Magnesium oxide, U. S. P. Baker's heavy powder; phenolsulfonphthalein (phenol red), Eastman Kodak Company; petroleum ether, Skellysolve F having a boiling range of about 30° to 60° C. The petroleum ether was distilled before use.

Fatty Acid Samples

Stearic acid, Eastman Kodak Company, m. p. 69.8° C.; myristic acid, Eastman Kodak Company, m. p. 54.6° C.; oleic acid, Baker's U. S. P.

Preparation of Impregnated Magnesium Oxide

About 0.5 gram of phenol red was dissolved in 10 to 15 ml. of 95 per cent alcohol. A few grams of magnesium oxide were added and stirred into a paste, which was mixed thoroughly

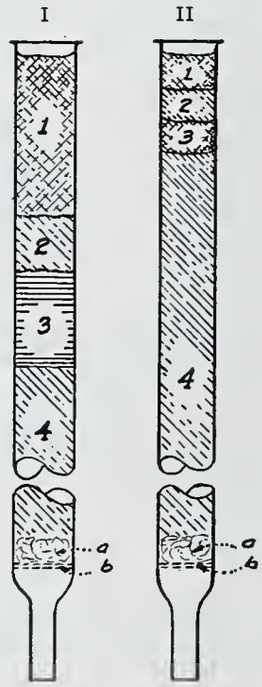

Figure 1. Absorption Columns

I, Oleic and stearic acids. II. Myristic and stearic acids.
a. Cotton plug. *b*. Perforated porcelain disk.

Zone	Length of Zone I	Length of Zone II
	Mm.	*Mm.*
1	35	8
2	12	7
3	21	7
4	125	175

	Color I	Color II
1	Yellow pink	Yellow pink
2	Original pink	Original pink
3	Light pink	Yellow pink
4	Original pink	Original pink

while the alcohol was being evaporated on an electric hot plate. The resulting pink powder was dried in an oven at 100° C. The dried powder was mixed with 100 grams of magnesium oxide and the mixture was screened through a 200-mesh sieve. The adsorbent was then ready for use.

Procedure

The adsorption column was retained in a vertical cylindrical glass tube 11 to 12 mm. in diameter and 60 to 70 cm. in length, constricted at the lower end. A plug of absorbent cotton held in position by a perforated porcelain disk was tamped over the constriction in the glass tube.

A mixture of dye-impregnated magnesium oxide and petroleum ether in the form of a slurry was poured rapidly into the tube and the adsorbent was allowed to settle as the solvent percolated out of the tube, forming a column 20 to 22 cm. in length, but the liquid was never allowed to fall below the surface of the adsorbent. The best columns were obtained when the adsorbent was allowed to settle under a layer of solvent for several hours.

The fatty-acid mixtures were dissolved in about 50 ml. of petroleum ether and added to the column. The column was then cautiously filled with petroleum ether and connected by means of a siphon to a reservoir-flask of petroleum ether at a higher level. When the fatty-acid mixture came in contact with the adsorbent, a bright yellow band was formed which separated into zones upon development. The chromatogram was developed by allowing petroleum ether to percolate through the adsorbent continuously until clearly defined zones were obtained. The volume of petroleum ether used and the rate of flow varied, depending on the packing of the column.

After formation of clearly defined zones, the column was permitted to run dry, forced from the tube, and then cut into sections as defined by the change in the color of the adsorbent. The cuts were made in order best to demonstrate the separation of the constituents of the mixture. The fatty acids were recovered from the adsorbent by dissolving the latter in hydrochloric acid and then extracting with diethyl ether. The ethereal extracts were washed free of indicator and acid by water in a separatory funnel and subsequently dried over anhydrous sodium sulfate and evaporated to dryness under a stream of nitrogen. The residues were dried in a vacuum over phosphorus pentoxide to constant weight. Samples were removed for iodine number (*9*) and melting point determinations.

Table I. Adsorptive Separation of a Mixture of Oleic and Stearic Acids (Commercial Grades)

Section	Weight of Fraction *Mg.*	Iodine No.	Melting Point ° *C.*
1	110.2	1.6	68–69
2	21.6	72.8	...
3	71.3	91.6	...
4	1.4	...	...

Experimental

By means of the above procedure it was possible to demonstrate a separation of (I) an unsaturated fatty acid from a saturated fatty acid of the same number of carbon atoms and (II) two saturated fatty acids differing in length of chain by 4 carbon atoms.

In (I) a mixture of 100 mg. of stearic acid ($C_{18}H_{36}O_2$), melting point 69.8° C., and 100 mg. of oleic acid ($C_{18}H_{34}O_2$), iodine number 86.4, was used. The development of the chromatogram required 17 days. The appearance of the column and the definition of the zones are sketched in Figure 1. The analytical data shown in Table I indicate an almost complete separation of the fatty acids in the mixture. Practically all of the stearic acid was retained in section 1 and the oleic acid had moved down the column.

The fractions recovered from sections 2 and 3 were pale yellow oils.

In (II) a mixture of 50 mg. of stearic acid ($C_{18}H_{36}O_2$), m. p. 69.8° C., and 50 mg. of myristic acid ($C_{14}H_{28}O_2$), m. p. 54.6° C., was used. The development of the chromatogram required 14 days. The appearance of the column and the definition of the zones are sketched in Figure 1 and the analytical data are tabulated in Table II.

The fractions recovered on evaporation of the ether solutions from the different sections to dryness, without recrystallization, gave successively lower melting points from the top to the bottom of the column, indicating that a separation had been accomplished. These fractions probably contained traces of solvent or other accumulated impurities. By recrystallization from acetone it was possible to show that the acids recovered from section 1 and section 2 consisted mainly of stearic acid and myristic acid, respectively.

The generality of the application of this method to chromatographic separation of a mixture of fatty acids is indicated by the fact that definite zones have been obtained with other mixtures of fatty acids. In the case of palmitic and stearic acids, where the length of chain differs by only two carbon atoms, zones were developed, but it was difficult to establish definite proof of a separation using melting points as criteria because of the proximity of their melting points.

Table II. Adsorptive Separation of a Mixture of Myristic and Stearic Acids (Commercial Grades)

Sections	Weight of Fraction *Mg.*	Melting Point ° *C.*	Melting Point after Recrystallizing 1st time ° *C.*	Melting Point after Recrystallizing 2nd time ° *C.*
1	44.7	56–57	63	68
2	36.5	51–52	54	54
3	31.5	44–46	..	..
4	0.8	...	..	..

Summary

A method has been described for separating mixtures of higher fatty acids by Tswett adsorption analysis, whereby separation into zones was observed on a column of heavy magnesium oxide impregnated with a suitable indicator. The fatty acids were recovered by dissolving the magnesium oxide in particular sections of the column in acid and extracting with ether. By this procedure it was possible to demonstrate a separation of an unsaturated fatty acid from a saturated fatty acid of the same number of carbon atoms and of two saturated fatty acids differing in chain length by four carbon atoms.

Acknowledgment

The authors wish to thank H. R. R. Wakeham for his interest and cooperation in the course of this work.

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Determination of Fluorine in Organic Compounds with Cerous Nitrate

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THE increasing importance of organic fluorine compounds makes a simple, reliable method for the determination of fluorine in such compounds extremely desirable. Reliable methods for the determination of chlorine, bromine, and iodine in organic compounds have been available for many years. However, fluorine differs considerably in its chemical behavior and the usual halogen methods cannot be applied for its determination.

Halogens are generally determined in organic compounds by decomposition of the compound and then determination of the halide ion. The fluorine-to-carbon bond is much more stable than the other halogen-to-carbon bonds and this makes the problem of decomposing organic fluorine compounds, to transform the fluorine to the fluoride ion, somewhat more difficult than in the case of the other halogen compounds.

In the case of compounds of the less stable type, where the fluorine is on a side chain or in certain aliphatic compounds, the decomposition may be accomplished by refluxing with metallic sodium or potassium in absolute alcohol or any other suitable inert solvent. Drogin and Rosanoff (*15*) applied this method for chlorine, bromine, and iodine, but Whearty (*59*) was not very successful in applying it to the analysis of several fluorine compounds. Vaughn and Nieuwland (*57*) used sodium in liquid ammonia or if the compound was insoluble in liquid ammonia, added ether. In a few cases where the carbon bonded to the fluorine is also bonded to oxygen, as with carbonyl fluorides, fluorinated alcohols, or sulfonyl fluorides, decomposition may be accomplished by refluxing with aqueous or alcoholic potassium hydroxide. This was used by Morgan and Tunstall (*42*) with boron beta-diketone fluorides.

Piccard and Buffat (*46*) heated fluorobenzene in a glass tube with potassium at 400° C. and obtained a rapid removal of the fluorine. Similarly Simons and Block (*53*) and Elving and Ligett (*17*) decomposed fluorocarbons and other fluorine compounds by heating with sodium or potassium in an evacuated tube.

Kimball and Tufts (*32*) recommended thermal decomposition. Although chlorine, bromine, and iodine can be transformed to free ions by this method at moderately high temperatures, Meyer and Hub (*40*) found that fluorine bonded to aromatic rings required 2 hours' heating at 1000° C. in a nickel or platinum bomb tube for complete transformation into the fluoride ion.

Bockemüller (*9*) considered the general methods of decomposition and used a combustion method. The fluorine compound was burned in an atmosphere of oxygen at 900° C. in a platinum tube with the exit end of the tube packed with granular calcium oxide. Hubbard and Henne (*30*) used a similar method in a silica tube packed with granular quartz. Bigelow (*8*) modified the method slightly. The method appears to be very useful for gaseous compounds and very volatile liquids, but has not been used for stable solid substances.

Another method which appears to have general application is sodium peroxide fusion using a Parr bomb (*36*, *45*). Hahn and Reid (*24*), Bigelow (*8*), and Locke, Brode, and Henne (*37*) have reported analyses of fluorine by this method.

After the fluorine is in the ionic state, it may be determined either gravimetrically or volumetrically. The gravimetric methods most generally used are those of Berzelius (*7*, *9*), Starck (*55*) and Hawley (*25*), Pisani (*47*), and Allen and Furman (*2*). Adolph (*1*) obtained reliable results with both the calcium fluoride method and lead chlorofluoride method, which was also used by Elving and Ligett (*17*), but the thorium fluoride method of Pisani gave low results. The triphenyl tin fluoride method of Allen and Furman is somewhat inconvenient, since the precipitation is made in boiling 70 to 80 per cent alcohol.

The most important volumetric methods are those of Willard and Winter (*60*), Kurtenacker and Jurenka (*34*), Batchelder and Meloche (*5*), Gyot (*23*), Greef (*22*), Offerman (*44*), Hempel and Scheffler (*26*), Wagner and Ross (*58*), and Hoffman and Lundell (*28*).

Willard and Winter titrated with thorium nitrate using a zirconium-alizarin mixture as indicator, and Langer (*35*) made this titration polarographically. Gyot and Greef titrated the fluorine with ferric chloride, using thiocyanate as indicator, and Treadwell and Köhl (*56*) determined this equivalence point potentiometrically. Hoffman and Lundell precipitated the fluorine as lead chlorofluoride, filtered, dissolved in nitric acid, and determined the chloride by the Volhard method.

Kurtenacker and Jurenka and Batchelder and Meloche proposed the titration of the fluoride with cerous nitrate. The titration must be started with the solution about neutral and at the equivalence point there is a rapid change in the pH which can be determined by using methyl red as indicator or potentiometrically (*3*). The titration has also been made with yttrium nitrate (*20*).

The remaining methods depend upon the evolution of fluorine as silicon tetrafluoride, absorbing it in water or alkali, and titrating. Comprehensive surveys of the methods of determining fluorine are given by Meyer (*39*) and Hernler and Pfeningberger (*27*).

Experimental

The titration of fluorine with cerous nitrate after the decomposition of the organic compound with sodium peroxide has the possibility of an accurate, rapid, and convenient method for the determination of fluorine but has been found to give erratic results. Therefore, this work was undertaken to make a systematic study of the effect of the initial pH, initial volume, concentration of fluorine, temperature, presence of neutral salts, and use of alcohol as solvent to determine the optimum conditions and limitations of this method.

Pure sodium fluoride was prepared by evaporating reagent hydrofluoric acid in a platinum dish to about three-fourths its volume, neutralizing with sodium carbonate solution, making just acid with hydrofluoric acid, and cooling. The sodium fluoride was filtered off on a sintered-glass funnel, washed with 50 per cent alcohol, dried at 110° C., and ignited to a dull red. This pure sodium fluoride was analyzed for fluorine by the lead chlorofluoride method (*25*) and three analyses gave results of 45.26 ± 0.24 per cent fluorine. A spectroscopic examination showed only a trace of silicon and less than 0.01 per cent of potassium.

Pure cerous nitrate was prepared by the method of Smith, Sullivan, and Frank (*54*). One thousand grams of 40 per cent crude ceric oxide were added slowly to 2 liters of hot concentrated nitric acid in a large evaporating dish on a steam bath. After all the oxide was added the gelatinous residue was filtered off, 400 grams of ammonium nitrate were added to the clear, deep red filtrate, and the solution was evaporated slowly. When the supernatant liquid became light yellow, the crystals of ceric ammonium nitrate were filtered off on a sintered-glass funnel. This salt was about 95 per cent pure and when ignited to the oxide was dark brown, which is characteristic of ceric oxide contaminated with other rare earth oxides (*18*). Five recrystallizations from hot concentrated nitric acid gave pure ceric ammonium nitrate. The absorption spectrum of a 0.3 *M* solution of this salt with a glass-prism Hilger spectrograph showed that praseodymium and neodymium were present in less than 2 parts per thousand (*4*, *10*, *49*, *50*).

The purified ceric ammonium nitrate was dissolved in water, reduced with perhydrol, and precipitated with oxalic acid. The cerous oxalate was washed several times by decantation with a hot, saturated ammonium oxalate solution, filtered, washed free from ammonium oxalate, and dissolved in nitric acid. It was necessary to digest the solution on a steam bath for some time to destroy the oxalate completely. The cerium was again precipitated with sodium carbonate and the cerous carbonate filtered and washed thoroughly with hot water. The cerous carbonate was added to enough water to form a slurry and nitric acid added in an amount not quite sufficient to dissolve

it. This is essential, as it is impossible to remove the nitric acid if an excess is added. The excess cerous carbonate was filtered off and the solution evaporated until it became sirupy. After crystallization, the cerous nitrate was removed by heating the dish for a few minutes, pulverized, and stored in glass-stoppered bottles.

The potassium dichromate was prepared according to Kolthoff and Sandell (*33*) and all other reagents were of reagent grade and used without further purification.

Approximately 0.01 *N* solutions of sodium fluoride, cerous nitrate, ferrous ammonium sulfate, and potassium dichromate were prepared. The sodium fluoride and potassium dichromate solutions were standardized by direct weighing, the ferrous ammonium sulfate by titration against dichromate using diphenylamine sulfonic acid, and the cerous nitrate by titration (*61*) against the ferrous ammonium sulfate, using *o*-phenanthrolineferrous ion.

FIGURE 1

Batchelder and Meloche (*5*) tried to carry out the titration of sodium fluoride with cerous nitrate potentiometrically but obtained unsatisfactory results due to adsorption by the hydrous cerous fluoride. However, a few preliminary experiments with the glass electrode showed that it gave reproducible results, and that a definite change in pH occurred at the equivalence point.

The glass electrode was of the bulb type, constructed from Corning 015 glass tubing, containing an internal silver–silver chloride electrode (*11, 13, 38*) immersed in 0.1 *N* hydrochloric acid. The calomel cell (*11*) was made from 12-mm. Pyrex tubing with a fine Pyrex sintered-glass plate sealed into the lower end. Over this was placed a 4-cm. layer of agar to reduce diffusion. The cell proper was made of 5-mm. soft-glass tubing containing a small hole for contact with the outer potassium chloride solution. The assembled electrode is shown in Figure 1. The electrometer used was a Leeds & Northrup, Model 7660, single vacuum tube instrument.

Since the glass electrode is subject to sodium-ion errors, some means of estimating or compensating for them was necessary. The original data of Dole (*11, 12*) and Jordan (*31*) on these errors were examined and by changing the constants in Jordan's equation to fit the data of Dole and Weiner (*14*) an empirical equation was found from which these errors could be calculated at sodium-ion concentrations of 0.1, 1.0, and 3.5 *M*. Using this equation, $\log \Delta E = 0.54\ \mathrm{pH}' + 0.60\ (\log \text{concentration } Na^+) - 4.35$, the agreement between calculated and experimental data was good and showed that this error did not exceed 3 millivolts up to a pH of 9.0 and a sodium-ion concentration of 1.0 *M* at 25° C. The sodium-ion errors are greatly affected by change in temperature (*21, 48*). The glass electrode was also calibrated and checked daily against buffer solutions at 25° C. to correct for asymmetry potential.

A study of the effect of the initial pH upon this titration showed that consistent results were obtained only when the initial pH was between 7.0 and 9.0. Under these conditions the calculated and theoretical volumes of cerous nitrate agreed within 4 parts per thousand.

A determination of the effect of the initial volume and the amounts of fluorine that can be determined was made by titrating solutions containing 1, 2, 4, 8, 16, and 30 mg. of fluorine with 0.01 *M* cerous nitrate at various dilutions. The solutions were always adjusted to a pH of 8 and dilutions giving a change of less than 7 millivolts at the equivalence point were not considered. The results for the titration of 2, 4, and 8 mg. of fluorine in 10 ml. are given in Table I and Figure 2. These data are the average of three titrations.

The results indicated that the amount of fluorine is best kept between 1 and 10 mg. and that the initial volume should be 10 to 20 ml. The theoretical volumes are based on independent standardization and the assumption that CeF_3 is formed.

Since the organic compounds are to be decomposed with sodium peroxide and then neutralized with nitric acid, the most important salt to be considered for the effect of neutral salts is sodium nitrate. This was determined by titrating 2 mg. of fluorine in 10 ml. of water at 25° C. in the presence of varying amounts of sodium nitrate. The sodium nitrate effectively reduced the magnitude of the change in potential at the equivalence point. A satisfactory change in potential at the equivalence point and stoichiometric results were obtained up to a concentration of 1.0 *M* in sodium nitrate, but at higher concentrations the volume of cerous nitrate used was about 1 per cent too small.

TABLE I. DETERMINATION OF FLUORINE

Cerous Nitrate Added *Ml.*	E. m. f.	Cerous Nitrate Added *Ml.*	E. m. f.	Cerous Nitrate Added *Ml.*	E. m. f.
0.00	0.3620	0.00	0.3600	0.00	0.3550
1.00	0.3000	3.00	0.2980	4.00	0.2960
2.00	0.2740	6.00	0.2360	8.00	0.2760
3.00	0.2250	6.20	0.2190	12.00	0.2250
3.10	0.2120	6.30	0.2060	12.50	0.2040
3.15	0.2040	6.35	0.2000	12.60	0.1960
3.19	0.1910	6.40	0.1880	12.70	0.1860
3.23	0.1780	6.44	0.1800	12.80	0.1730
3.28	0.1720	...	....	12.90	0.1660
Fluorine present, mg.	2.0		4.00		8.00
Cerous nitrate used, ml.	3.18 ± 0.02		6.37 ± 0.03		12.75 ± 0.05
Cerous nitrate, theoretical, ml.	3.17		6.35		12.70
pH at equivalence point	5.10		5.19		5.02

Sodium chloride, bromide, and iodide showed the same effect but, in general, the amount of these salts introduced from the organic compounds will be small and their contribution to the total salt concentration can be neglected. Sulfates interfered badly and must be absent. The presence of 2 mg. of sodium sulfate in 10 ml. made it impossible to detect the equivalence point. Perchlorates interfered seriously when there was more than 0.04 gram of sodium perchlorate in 10 ml. The presence of perchlorates did not elimi-

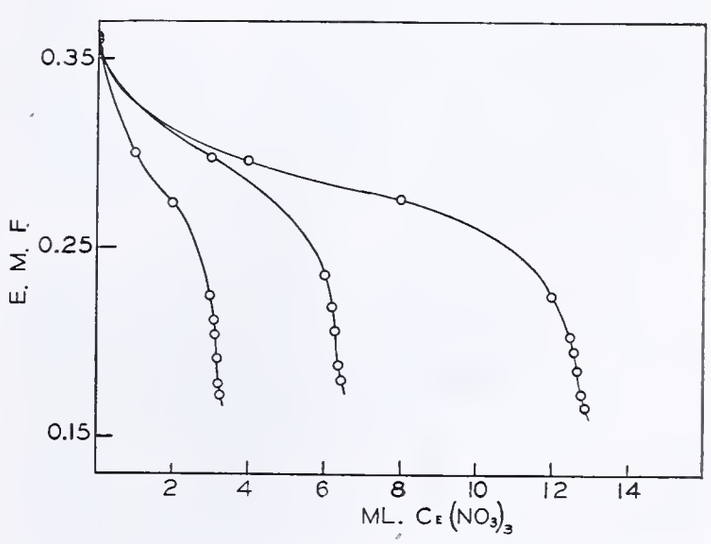

FIGURE 2

nate the potential change at the equivalence point but displaced it so as to give high results. Carbonates interfered, giving high results due to the precipitation of the cerous ion, but can be removed by careful neutralization and boiling. Any other ion which acts as a buffer or precipitates either fluoride or cerous ion will also cause trouble, but the above ions are the only ones which will ordinarily be encountered.

Kurtenacker and Jurenka (*34*) recommended that the titration be made at 80° C. The effect of temperature was examined by comparing the titration of like amounts of fluorine at 25° and 90° C. The change in e. m. f. at the equivalence point at the higher temperature was about twice that at 25° C. and the equilibrium was established much more rapidly, but the volume of cerous nitrate used was about 1 per cent too large. While the titration gave good results with pure solutions, the presence of neutral salts presented a serious interference, especially with visual titrations. However, this effect was lessened by titration in hot solution.

If 50 per cent ethyl alcohol was used as the solvent and the titration made at 90° C. the change in pH at the equivalence point was increased again to a surprising degree and equilibrium was quickly established. This was shown by two titrations of 1.5 mg. of fluorine in the presence of 1.7 grams of sodium nitrate at 90° C. in 20 ml. of water and in 20 ml. of 50 per cent alcohol. Both solutions were adjusted initially to a faint pink color with phenolphthalein. The change in e. m. f. at the equivalence point with alcohol was twice that with water, but the volume of cerous nitrate used was 5 per cent higher than the theoretical volume with water. No satisfactory means exists of correlating the e. m. f. of the glass electrode with the absolute pH in nonaqueous solvents but it can be used to follow changes in pH, as has been shown by Schicktanz and Etienne (*51*) and Evans and Davenport (*19*). These titrations indicated that, if a compensation is made for the nontheoretical relationship by standardizing the cerous nitrate solution under the same conditions, the addition of ethyl alcohol is desirable whenever appreciable amounts of sodium nitrate are present.

Decomposition of Compound

An attempt to devise a general method for the decomposition of stable, solid, organic fluorine compounds on the combustion method of Hubbard and Henne (*30*) was unsuccessful. The results obtained were invariably low.

Although no single comprehensive investigation has been made to test the general applicability of the Parr bomb fusion for the decomposition of organic fluorine compounds, the work of Hahn and Reid (*24*), Bigelow (*8*), Locke, Brode, and Henne (*37*), and Miller (*41*) on individual compounds indicated that it might be suitable. Elek and Hill (*16*) and Beamish (*6*) used this method for the other halogens, but their procedure failed to give complete combustion of 25 to 50 mg. of fluorine compounds. The following procedure, however, was found to give satisfactory results.

About 20 to 50 mg. of the compound, representing at least 5 mg. of fluorine, were taken. Solid compounds must be finely ground and can be weighed in small weighing tubes (*43*). Liquids or low-melting solids can be handled most conveniently in the small, No. 1, gelatin capsules of Parke, Davis and Co. The charge was made up of 3 grams of sodium peroxide, 100 mg. of sugar, and 300 mg. of potassium nitrate, finely ground, thoroughly mixed, and stored in a glass-stoppered vial. When solids were analyzed the weighed, finely ground sample was placed in the nickel microbomb cup, one-half the charge added, thoroughly mixed, and the rest of the charge added. With liquids, a small amount of the charge was placed in the bomb, and the closed capsule containing the liquid was seated in the center of the charge, pressed to the bottom of the cup, and then covered completely with the peroxide charge. Normally the complete charge was not used, but the cup should be filled to within about 0.3 cm. (0.125 inch) of the top. The lid was clamped firmly in position, the bottom of the bomb was held in a strong Bunsen flame, and after a disturbance was felt the bomb was cooled with tap water. If no disturbance was felt the bomb was heated about 20 to 30 seconds before cooling. With the semimicrobomb a sample of 100 to 150 mg. was used with a charge of 8 to 9 grams of sodium peroxide and 300 mg. of sugar. The bomb was heated over a strong Bunsen flame or Fisher burner until the water in the cooling ring evaporated.

After the bomb was cooled and rinsed, the contents were dissolved in a small amount of water in a 125-ml. Erlenmeyer flask and the solution was boiled to destroy the peroxide. A slight residue of carbon apparently does not affect the accuracy of the determination. The solution was adjusted to the pale pink color of cresol red with strong nitric acid and sodium hydroxide and heated, until the color no longer changed on boiling, to remove the carbon dioxide.

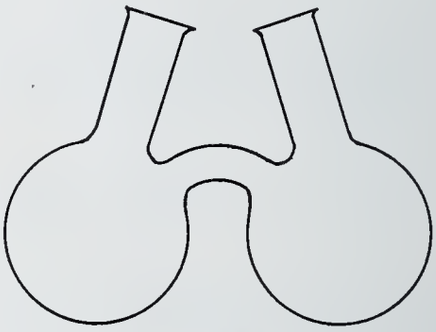

Figure 3

Practically all the nickel precipitated during this process. The nickel precipitate was filtered off and washed thoroughly, the filtrate was adjusted to a volume of 20 to 30 ml., and the neutralization was completed with 0.1 *N* nitric acid. During this neutralization the solution should be alternately adjusted to a faint pink color and boiled until 3 to 4 drops of the nitric acid change the color to a clear yellow and the color remains unchanged after 2 minutes' gentle boiling. The solution was cooled and transferred directly to a 50-ml. measuring flask, if no more nickel had precipitated; otherwise it was filtered again, as the titration fails in the presence of any nickel precipitate. The solution was diluted to 50-ml. and 10-ml. aliquot portions were taken for the titration.

Attempts to carry out the decomposition by the above procedure in a steel semimicrobomb were unsuccessful, as the iron was not removed completely. Some further modification, which will completely remove the iron, is necessary before this procedure can be used with a steel bomb.

The fluoride ion may now be titrated either electrometrically or visually.

Electrometric Titration

The 10-ml. portion of the neutralized solution of fluoride, containing between 1 and 6 mg. of fluorine so as to be able to titrate with a 10-ml. buret, was placed in a 50-ml. beaker. The concentration of sodium nitrate should not exceed 2 *M*. Ten milliliters of alcohol were added and the beaker was placed on a small electric hot plate clamped to a ring stand. A small electric glass stirrer and the electrodes were introduced into the solution. While the solution was heating, one drop of phenolphthalein was added and the solution was adjusted with 0.001 *N* sodium hydroxide and nitric acid to a faint pink color. After the solution began to boil, the hot plate was adjusted to low heat and the pH of the solution readjusted if necessary. When the initial e. m. f. had become steady the fluorine was titrated with 0.01 *N* cerous nitrate until the maximum change in e. m. f. per unit volume of cerous nitrate was obtained.

Table II. Analysis of Organic Fluorine Compounds

Compound	Fluorine Theoretical	Visual Titration No. of detns.	Visual Titration Fluorine found	Electrometric Titration No. of detns.	Electrometric Titration Fluorine found
	%		%		%
4,4-Difluorodiphenyl	20.00	4	19.9 ± 0.2	1	19.7
Fluorobenzoic acid	13.57	2	13.75 ± 0.05	1	13.6
Fluorobenzene	19.80	2	19.6 ± 0.1	1	19.6
2,5-Dichloro-1-fluorobenzene	10.62	2	10.85 ± 0.05	2	10.7 ± 0.1
Octachlorodifluorobutane	10.27	1	10.3	2	10.1 ± 0.0
Tetrachlorodifluoroethane	18.62	2	18.65 ± 0.05	2	18.7 ± 0.1
Trichlorotrifluoroethane (Freon)	30.30	2	30.0 ± 0.0	2	30.2 ± 0.0
Benzotrifluoride	39.03	1	39.1	2	39.1 ± 0.1
Nitrobenzotrifluoride	29.80	2	29.9 ± 0.1	3	29.6 ± 0.1

Visual Titration

The titration of pure sodium fluoride with cerous nitrate using methyl red gives little trouble when the solution is kept near the boiling point. However, the presence of neutral salts such as sodium nitrate in 1 molar concentration in aqueous solution makes the equivalence point very difficult to detect and renders the titration practically useless. Under the same conditions as used for the electrometric titration the visual titration with methyl red gave satisfactory results.

For this titration, 0.1 *N* and 0.001 *N* solutions of both nitric acid and carbonate-free sodium hydroxide, a special titration flask, a flashed opal screen with a daylight light bulb, a saturated solution of methyl red in ethyl alcohol, a 0.02 per cent solution of cresol red, and a 0.04 per cent solution of bromocresol green are required.

The titration flask (Figure 3), suggested and designed by W. T. Miller, is made of two 200-ml. round-bottomed Pyrex flasks, connected with a short curved tube which allows the solution to be poured back and forth between the two flasks. The complete titration must be carried out in front of the flashed opal screen, as the color changes of the indicators are too delicate to be observed clearly in ordinary light.

A 10-ml. aliquot portion of the neutral fluoride solution was placed in one side of the double flask and 10 ml. of 95 per cent alcohol were added. Three drops of cresol red were added, and the solution was adjusted to a definite pink color, and then brought to a gentle boil. The solution was then made acid with 1 or 2 drops of 0.1 *N* nitric acid in excess and the solution was boiled gently for 2 minutes, to remove all carbon dioxide. If the solution remains clear yellow during this period, the carbonate has been removed. The solution was then adjusted to a very faint pink color with the 0.001 *N* solutions of acid and base. The solution should be poured freely from one side of the double flask to the other, so that it remains constant in composition.

Two drops of the saturated methyl red and one drop of bromocresol green were added. This mixed indicator was recommended by Hubbard and Henne (*30*). The initial color of the solution should be a clear green. The solution was now titrated drop by drop, being boiled and agitated at frequent intervals. When the color became a light orange, the solution was divided evenly between the two flasks and one more drop of cerous nitrate added to one portion of the solution. The color difference between the two halves was noted. The two portions were reunited, poured back and forth several times, brought to a boil, and divided equally again. Another drop of cerous nitrate was added and the difference in color again noted. This procedure was repeated until a maximum color difference was observed.

The results in the previous sections confirm those of Kurtenacker and Jurenka that when fluoride, in hot 50 per cent alcohol and in the presence of sodium nitrate, is titrated with cerous nitrate either with a glass electrode or with an indicator, the result is not the stoichiometric relationship found in pure aqueous solution. Therefore, the cerous nitrate solution must be standardized against a known amount of sodium fluoride under the same conditions of concentration of sodium nitrate, temperature, and solvent. The potassium nitrate in the fusion mixture and any halogen contained in the organic compound may be neglected with regard to their contribution to the total salt concentration.

Using the above outlined procedure nine organic fluorine compounds were analyzed. The results obtained are given in Table II and in many cases are the average of several analyses on varying weights of samples.

Since the fluoride ion cannot be titrated with cerous nitrate in the presence of the sulfate ion, attempts were made to modify the proposed procedure to secure a separation. The distillation from perchloric acid, as advocated by Willard and Winter (*60*), was tried with *p*-methylbenzenesulfonylfluoride after decomposition in a microbomb, but low results were always obtained. Evidently the presence of a large amount of sodium perchlorate makes the quantitative recovery of fluorine impossible. Others have reported this same difficulty with natural waters when the salt concentration was appreciable. However, Scott and Henne (*52*) claimed a successful distillation from biological material ashed with calcium oxide.

Attempts to separate the sulfate ion by precipitation as barium sulfate always gave low results, probably because of the precipitation of barium fluoride in the 50 per cent alcohol solutions. Hoskins and Ferris (*29*) reported the same difficulty in the titration of fluorine with thorium nitrate in the presence of the barium ion.

The best method, at present, for determining fluorine in the presence of sulfate appears to be that of Starck (*55*) and Hawley (*25*) with the titration by the Volhard method (*28*). An analysis of *p*-methylbenzenesulfonylfluoride by this method gave 11.2 ± 0.1 per cent fluorine.

Two different explanations have been offered for the observed color change of methyl red in this titration. Kurtenacker and Jurenka (*34*) stated that this is due to a change in pH at the equivalence point caused by the hydrolysis of the excess cerous nitrate. Batchelder and Meloche (*5*) think that the methyl red behaves as an adsorption indicator. During the titration there is a definite flocculation of the gelatinous cerous fluoride precipitate and these experiments have shown that a clearly defined change in pH occurs at the equivalence point. However, the pH change obtained is somewhat greater than can be accounted for on the basis of hydrolysis, even assuming that the cerous ion undoubtedly exists as a coordination complex. Moreover, considering the effects of alcohol and temperature, it is evident that a rather complex condition exists and neither of the above explanations is entirely adequate.

Summary

The effects of the initial pH, initial volume, temperature, and the presence of alcohol and several neutral salts, especially sodium nitrate, on the titration of fluoride ion with cerous nitrate have been studied.

The equivalence point of the titration can be determined either electrometrically with a glass electrode or visually with methyl red as indicator. This titration in general is capable of yielding results with an accuracy of ±1 per cent. The electrometric determination is superior and should be employed where the highest possible accuracy is desired. However, when little or no neutral sodium salts are present, the visual titration is satisfactory and yields results which compare favorably with the electrometric determination.

The determination of fluorine, in both aliphatic and aromatic compounds, based on the decomposition of the compound by the sodium peroxide fusion in a nickel Parr microbomb and titration with 0.01 *N* cerous nitrate has been outlined. The accuracy of the determination is ±1 per cent.

Sulfate and perchlorate ions interfere. The titration of fluorine with cerous nitrate cannot be carried out in the presence of the sulfate ion. An alternate method for this type of compound has been suggested. The presence of perchlorate is neither desirable nor necessary.

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Extraction and Colorimetric Estimation of Certain Metals as Derivatives of 8-Hydroxyquinoline

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ALTHOUGH methods involving the use of 8-hydroxyquinoline as a quantitative precipitant have been extensively investigated and widely used (*5*), but little attention has been devoted to colorimetric estimations based upon this reagent. The lack of general applicability of colorimetric procedures is probably traceable to the complexity or indirectness of those methods which have been suggested.

Among the colorimetric methods proposed are the following:

Aluminum and magnesium through coupling of the 8-hydroxyquinoline derivatives with sulfanilic acid, followed by colorimetric estimations in terms of the dyes so produced (*2*, *3*); bismuth through extraction of the 8-hydroxyquinoline complex with a 3 to 1 acetone–amyl acetate mixture and color comparison of the extract (*19*); iron through solution of the precipitated ferric complex in ethanol followed by colorimetric observation of the resulting solution (*15*); magnesium (*6*, *22*), calcium (*6*, *22*), and bismuth, aluminum, and zinc (*21*) through the colors produced by reactions of the 8-hydroxyquinoline derivatives with Folin's reagent (*10*); magnesium by addition of excess 8-hydroxyquinoline and colorimetric estimation of the excess after removal of the precipitated magnesium complex (*14*); magnesum by development of color through reaction of the precipitated derivative with ferric chloride in acetic acid (*12*); and iron (*1*), aluminum (*1*), and indium (*16*) through extraction of the colored complexes with chloroform and direct colorimetric observation of the diluted extracts.

For simplicity of operation, the last of these methods would appear particularly advantageous, consisting as it does in a mere extraction of an aqueous solution, adjusted to an appropriate pH value, with a solution of 8-hydroxyquinoline in chloroform, layer separation, and observation of the spectral transmittancy of the extract at a predetermined wave length. The sensitivity of the method for the accurate estimation of small quantities of iron, aluminum, and indium (*1*, *16*) has been demonstrated.

It is the purpose of this paper to present data relative to the necessary conditions for the extraction of the complexes of cobalt, nickel, bismuth, and cupric copper, spectrophotometric data for solutions of these complexes in chloroform and data relative to the possible estimation of small amounts of these elements by this procedure. Inasmuch as a reinvestigation of the method as applied to iron and aluminum has yielded results significantly different from those reported

by Alexander (*1*), it has been deemed advisable to include these elements as well.

Apparatus and Materials

All colorimetric measurements were made with the Cenco-Sheard spectrophotelometer (*20*) previously employed (*16*), using cells of 1-cm. (±0.5 per cent) thickness with a slit width of 5 mμ. Measurements of pH were made with a Beckman Laboratory Model G pH meter (*16*).

Chloroform and 8-hydroxyquinoline were of the same purities as previously reported (*16*), and chloroform was recovered by the same procedure (*16*), except that 12 *N* hydrochloric acid proved to be a better extracting agent. All other chemicals were of analytical reagent quality and were used without further purification.

Spectrophotometric Examination of Chloroform Solutions of 8-Hydroxyquinoline Derivatives

Since in a preliminary investigation it was found that of the 8-hydroxyquinoline derivatives of aluminum, ferric iron, cupric copper, bismuth, cobalt, and nickel prepared and dried by the recommended methods (*5*) only the first three dissolved at all readily in chloroform, spectrophotometric studies were made upon solutions of these materials as extracted from aqueous salt solutions at appropriate pH values.

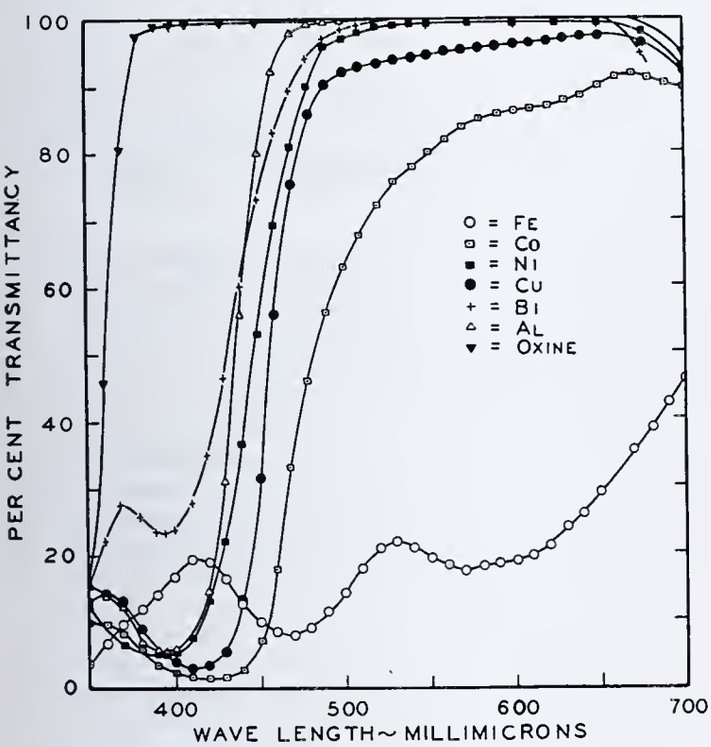

Figure 1. Transmittancy Curves for Chloroform Solutions of 8-Hydroxyquinoline and Certain Metallic Derivatives

In Figure 1 are given curves showing the spectral transmittancies of these complexes over the wave length range 350 to 700 mμ. In all cases except ferric iron, solutions contained the equivalents of 20 mg. of the metals per liter of chloroform; for ferric iron it was 10 mg. Included for purposes of comparison is a curve obtained for a solution prepared by dilution of 20 ml. of 0.01 *M* 8-hydroxyquinoline ("oxine") in chloroform to 50 ml.

Although the absorption bands for the metallic derivatives are all broad, maximum absorption is noted definitely at the following wave lengths: iron, 470 and 570 mμ; aluminum, bismuth, and nickel, 395 mμ; cobalt, 420 mμ; and copper, 410 mμ. Furthermore, in the region around 400 mμ, chloroform solutions of 8-hydroxyquinoline, although apparently

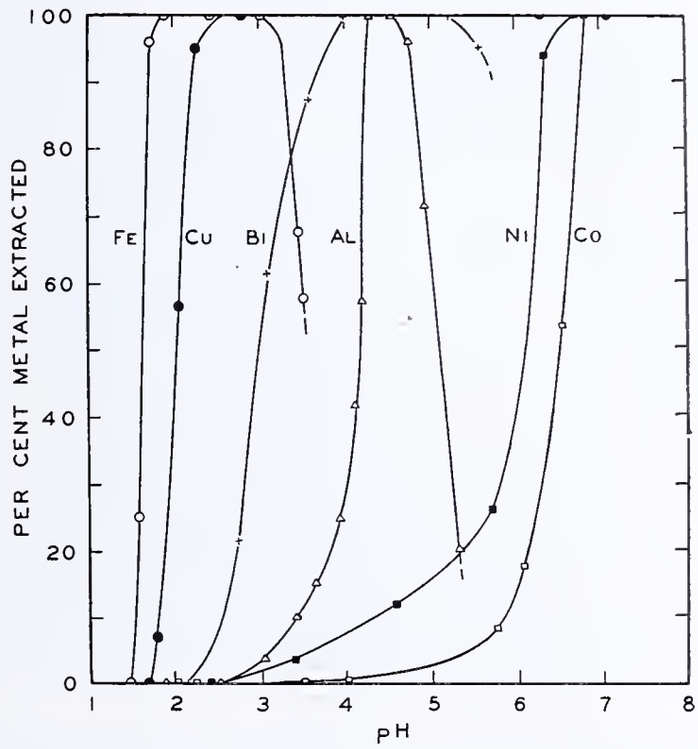

Figure 2. Effect of pH upon Metal Extraction

colorless, show measurable absorptions (*1*). However, as has been pointed out (*1*, *16*), the use of constant and controlled conditions of extraction obviates this difficulty.

Extraction and Colorimetric Estimation

Extraction of metallic ions from aqueous solutions as derivatives of 8-hydroxyquinoline is as closely dependent upon pH (*1*, *16*) as is their precipitation by that reagent (*8*, *9*, *13*). Optimum pH ranges for extraction were determined as previously reported (*16*), using nitrate, sulfate, or chloride solutions containing in 25 ml. the equivalent of 20 mg. (10 mg. for iron) of the metal per liter of chloroform and adjusted to appropriate pH values with 0.1 *N* sulfuric acid or sodium acetate, extracting four times with 5-ml. portions of 0.01 *M* 8-hydroxyquinoline, and examining the extracts at appropriate wave lengths after dilution to 50 ml., the per cent extraction then resulting from application of calibration curves (Figure 3). Data so obtained are plotted in Figure 2.

Transmittancy data obtained at the wave lengths previously given for extracts made within the optimum pH ranges indicated in Figure 2 and containing the equivalent of 5, 10, 15, and 20 mg. of metal per liter of chloroform are plotted in Figure 3. Lack of intersection with the logarithm of 100 per cent transmittancy at zero concentration is due to the measurable transmittancy of the reagent in chloroform.

Iron. Data in Figure 2 confirm the observation of Alexander (*1*) that ferric iron is completely extracted at pH 2.0, although complete extraction was obtained here at pH 1.9. Inasmuch as completeness of extraction falls off above pH 3.0, owing to hydrous oxide formation (*16*), optimum conditions for the removal of iron are in the pH range 1.9 to 3.0.

Although Alexander (*1*) reported the colorimetric estimation of iron at 540 mμ, data in Figure 1 indicate the greater desirability of using the 470 and 570 mμ bands. At either of these wave lengths, the color system obeys Beer's law rigidly in a concentration range at least up to 20 mg. of the metal per liter of chloroform (or up to 1 mg. of iron in 25 ml. of aqueous solution), although absorption at the lower wave length is so strong as to make reading difficult at higher concentrations (Figure 3). The extensive absorption of these black solutions over the entire visible spectrum (Figure 1) coupled with the low extraction pH

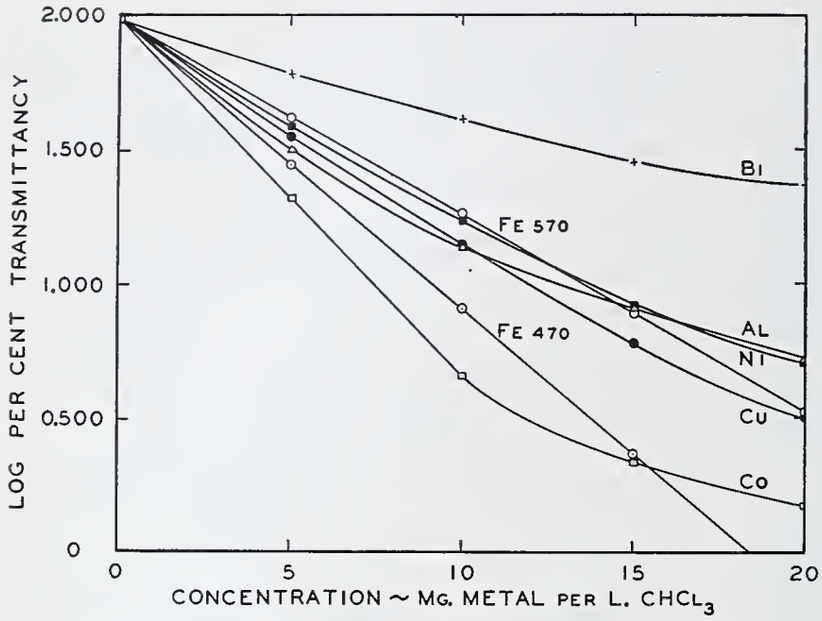

FIGURE 3. RELATION BETWEEN METAL CONCENTRATION AND TRANSMITTANCY

would cause ferric iron to interfere with the determination of certain other metals—e. g., copper, bismuth, and indium (*16*).

ALUMINUM. Although Alexander (*1*) reported complete extraction of aluminum at pH 3.5, data in Figure 2 indicate complete removal only in the pH interval 4.3 to 4.6, extraction again falling off at higher pH values. Partial extraction was noted in the pH interval 2.6 to 4.3, and within the optimum range complete extraction was attained only with difficulty. The use of higher reagent concentrations might improve the results.

Strict conformity to Beer's law at 395 mμ is not shown by chloroform solutions of the aluminum complex, although deviation in the range up to 20 mg. is insufficient to interfere with the method. Estimation of iron and aluminum in the same sample as reported by Alexander (*1*) appears to offer no difficulty, since iron extracts completely at pH values below those for aluminum extraction.

BISMUTH. Extraction of the yellow bismuth complex is complete in the pH range 4.0 to 5.2 and proceeds readily. At higher pH values, incomplete extraction is again noted. Reference to Figure 3 indicates but little deviation (at 395 mμ) from Beer's law up to 10 mg. of the metal per liter of chloroform, although differences become more marked at higher concentrations. Use of this method for the estimation of bismuth should present no difficulties.

COBALT. Although extraction of the cobalt complex begins around pH 3.5, complete removal occurs only at pH 6.8 or above. At appropriate pH values, extraction offers no difficulties, and complete removal of cobalt from the aqueous layer can usually be effected by two extractions. At 420 mμ, the slightly orangish chloroform solutions of the complex conform exactly to Beer's law up to 10 mg. of the metal per liter, but deviations at higher concentrations are marked.

NICKEL. Extraction of the nickel derivative begins just above pH 2.5 but is complete only at pH 6.7 or above. Deviations of the yellow chloroform solutions of the complex from Beer's law at 395 mμ are minor up to 10 mg. of the metal per liter but become more pronounced at higher concentrations. Quantitative estimation of nickel by this method should offer few difficulties.

COPPER. As is shown in Figure 2, extraction of the copper complex was complete at all investigated pH values in the range 2.7 to 7.0. Qualitative observations have indicated the complete removal of copper from ammoniacal solutions as well, an indication of the stability of the resulting compound.

At 410 mμ, the yellow chloroform solutions of the copper derivative approach Beer's law closely up to concentrations of 10 mg. of the metal per liter, with deviations becoming somewhat greater at higher concentrations.

Estimation of copper by this method is readily and accurately effected in the absence of other extractable cations, and the wide pH range of copper extraction permits a considerable choice of suitable pH values. Inasmuch as the estimation of copper in the presence of cadmium and zinc is often necessary and the cadmium and zinc derivatives of 8-hydroxyquinoline have been found not to extract at least below pH 5.5 (*18*), it was of interest to apply the above procedure to copper-cadmium and copper-zinc solutions. The accuracy and feasibility of the estimation of small amounts of copper in the presence of at least forty times as much of these elements are in dicated by data obtained by the extraction of 25-ml. aqueous sulfate solutions adjusted to pH 4.0 and containing in all cases the equivalent of 10.0 mg. of copper per liter of chloroform plus a varying quantity of the other metal. The transmittancy of the solution containing copper (at 410 mμ) was unaffected by as much as 400 mg. of cadmium or zinc. It appears not unlikely that the method would be applicable in the presence of even larger amounts of cadmium or zinc.

Although all the elements except iron here investigated give 8-hydroxyquinoline derivatives the chloroform solutions of which show maximum absorption in the region around 400 mμ, thereby imparting to the method a lack of specificity, it is apparent that through proper pH control and in the absence of interfering cations a method is available for the accurate determination of iron, aluminum, bismuth, cobalt, nickel, and copper in amounts up to at least 1 mg. in 25 ml. of aqueous solution. In those instances where one metal can be completely extracted at a pH below that at which a second metal extracts, separate determinations of the two are possible. This has already been reported for iron and aluminum (*1*) and the data here presented indicate that it should be possible to estimate iron in the presence of bismuth, cobalt, or nickel; copper in the presence of cobalt and possibly in the presence of nickel or aluminum; and copper in the presence of cadmium or zinc.

Extraction of metallic derivatives of 8-hydroxyquinoline and their precipitation from aqueous solution appear, in several instances, to be closely related to hydrous oxide or hydroxide precipitation. This is brought out by the pH data summarized in Table I, from which it is apparent that pH values for these three phenomena are most closely related with ferric iron, indium, aluminum, and bismuth. With copper, quinolate precipitation and extraction occur at a lower pH than hydrous hydroxide formation, while with cobalt and nickel, quinolate extraction and hydrous oxide formation agree but are at higher pH values than quinolate precipitation. Further investigations along these lines should be profitable.

TABLE I. pH RELATIONS BETWEEN HYDROUS OXIDES (HYDROXIDES) AND 8-HYDROXYQUINOLINE DERIVATIVES

Ion	pH Value: Hydrous oxide (hydroxide) formation	Quinolate precipitation	Quinolate extraction
Fe^{+++}	2 (*7*)	2.8 (*13*)	1.9
Cu^{++}	5.3 (*7*)	2.7 (*13*)	2.7
In^{+++}	3.4 (*17*)	3.0 (*11*)	3.2 (*16*)
Bi^{+++}	4.2 [a]	4.8 (*13*)	4.0
Al^{+++}	4.1 (*7*)	4.2 (*13*)	4.3
Ni^{++}	6.7 (*7*)	4.6 (*13*)	6.7
Co^{++}	6.8 (*7*)	4.2 (*13*)	6.8

[a] Calculated from solubility product data at 0.1 *M* (*4*).

Summary

Spectrophotometric examinations of chloroform solutions of certain metal derivatives of 8-hydroxyquinoline indicate the following absorption maxima: ferric iron, 470 and 570 mμ; aluminum, bismuth, and nickel, 395 mμ; cobalt, 420 mμ; and copper, 410 mμ.

Agitation of aqueous solutions containing these metallic ions with a chloroform solution of 8-hydroxyquinoline leads

to complete extraction of the metallic complexes in the following optimum pH ranges: iron, 1.9 to 3.0; aluminum, 4.3 to 4.6; bismuth, 4.0 to 5.2; cobalt, 6.8 or above; nickel, 6.7 or above; and copper, 2.7 to at least 7.0. At pH values below the lower limits, partial extraction to no extraction will occur.

Chloroform solutions containing the equivalents of up to 20 mg. of these metals per liter (or 1 mg. of the metal in the aqueous solution extracted) show little or no deviation from Beer's law at the wave lengths given and thus lend themselves to use in accurate colorimetric determinations.

Copper can be determined in the presence of at least forty times as much cadmium or zinc by extraction at pH 4.0 and transmittancy measurement at 410 mμ.

An interesting correlation between pH values for hydrous oxide or hydroxide formation, quinolate precipitation, and quinolate extraction is given.

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An Improved Meter for the Measurement of Gas Flow Rates

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IN THE study of reaction kinetics in flow systems, accurate measurement of the rate of flow of the intake and exit gases is essential. Flow-measuring instruments in common use are of two types: (A) meters that measure some function of the gas velocity—for example, the pressure drop across an orifice or the rate at which heat is conducted away from a heated wire—and (B) instruments that involve displacement of a liquid by the gas and continuous or discontinuous measurement of the volume displaced in unit time. The familiar wet-test meter is an example of this type. Both types of instruments, as commercially available, have disadvantages, particularly if flow rates of the order of 25 cc. per minute or smaller are to be measured. Type A requires a separate calibration curve for each kind of gas, hence is not suited for use with mixtures of unknown composition, such as the gaseous product of a thermal reaction. Type B requires estimation of and correction for the effect of the vapor pressure of the displaced liquid and for the solubility of some components of the mixture in the liquid. Meters of this type are therefore unsuitable for the measurement of intake rates.

The flowmeter shown diagrammatically in Figure 1 is an improved form of a meter described by Marsh (*1*) for measurement of very slow flow rates. The new type of meter is free from the disadvantages of the usual instruments in common use. The operation is clearly shown by the diagram.

Referring to the side view, gas enters at *A*, passes down the vertical tube, and forces a drop of mercury, *D*, to the right in the horizontal tube. Stopcocks S_1 and S_2 are open; S_3 is closed. When the drop reaches the end of the tube at *E*, the gas trapped behind the drop is discharged. However, as soon as the drop

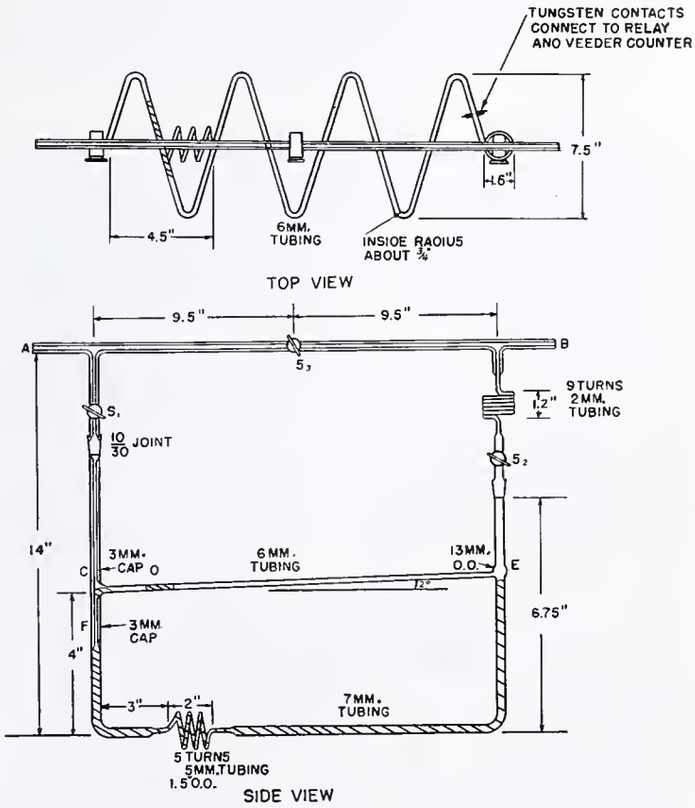

FIGURE 1. DIAGRAM OF FLOWMETER

begins to flow into the 13-mm. bulb at *E*, the mercury level is pushed up at *C*, so that a new drop is forced into the horizontal tube, trapping a new segment of the gas stream. This drop then moves to the right and the cycle is repeated.

Thus, each cycle represents the flow of a definite volume of gas and the time between cycles is a measure of the flow rate. For low flow rates the time required for movement of the drop between two fixed points may be a more convenient measure of the rate.

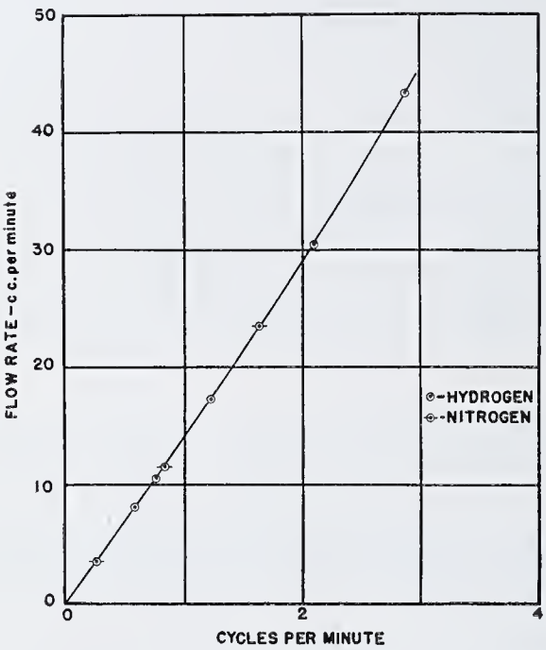

FIGURE 2

Figure 2 shows a calibration curve obtained with hydrogen and with nitrogen, when gas volumes were measured by collection over water in suitable burets. The points for the gases are seen to fall accurately on the same line. The use of mercury as a displacement fluid eliminates the difficulties caused by use of water or organic liquids in the usual wet-test meters.

Some details of the construction and operation of the flowmeter need further discussion. The glass spirals permit small adjustments in the tilt of the tube, which controls the size of the drop. Since there is a lag between the time at which the drop begins to flow into the bulb at *E* and the time at which the mercury rises at *C*, the drop must be of sufficient length to ensure that mercury will enter the horizontal tube at *C* before the drop at the right is entirely discharged from the tube. If this condition is not met, a jet of gas will flow through the tube during a part of each cycle. The amount of gas will be a function of the gas viscosity and the calibration curve will then be found to depend on the kind of gas.

The requirement that there must be a drop of mercury in the horizontal tube at every part of the cycle suggests the use of a tube of relatively large diameter and as short as possible for the mercury piston, in order to reduce the time lag. However, in such a tube the mercury level tends to oscillate for some time after a drop is discharged at *E*, thereby producing a series of drops at the left instead of one. The constriction in the vertical tube at *F* reduces these oscillations; the long zigzag horizontal tube, shown in the top view, ensures complete damping of the oscillations of one cycle before the second begins and minimizes possible errors from fluctuations in the size of the mercury drops. In the authors' instrument 2 drops are formed at the beginning of every cycle when the flow rate becomes greater than 15 cc. per minute and as this rate is approached there will be 1 drop one cycle and 2 the next. However, the total volume of mercury remains very nearly constant, as the calibration curve proves. Above 35 cc. per minute 3 drops are formed. The tungsten contacts shown in the top view actuate a counter through a relay, so that the instrument may be used to record total flow as well as to measure the rate.

The surface of the tubing through which the gas and mercury droplets flow should be free of any oil or water film or dust particles, and the mercury should be pure; otherwise there will be small fluctuations in the rate of gas flow which may give pressure variations as high as 1 or 2 mm. of mercury. This effect can be eliminated in any case, however, by having a surge chamber in the line following the meter.

Since the volume displacement per cycle does not depend on the pressure or temperature of the gas, the instrument may be used at subatmospheric pressures. During any particular run, however, the temperature and pressure should have constant values; otherwise it would be difficult to convert the gas volumes to standard conditions. The meter could also be adapted for use at superatmospheric pressures by using metal parts or suitable plastic tubing and connections.

Acknowledgment

The authors are greatly indebted to Harold Gershinowitz for his interest and advice.

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Forceps with Platinum-Covered Tips

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THE usual platinum-tipped forceps commercially available have each platinum tip fastened to nickel-plated steel by means of two rivets in a simple scarf joint. During the past five years, three or four of these expensive tools have failed here, always owing to corrosion or oxidation where the two metals join. Even moderate attack of the steel soon renders the forceps unfit for use because the platinum alone is too weak to withstand the flexing that occurs at the rivet holes.

Forceps that are stronger, cheaper, and more durable than those just described have been made in this laboratory by covering the tips of ordinary steel forceps with 5-mil platinum foil. The tips of the steel forceps were filed to the shape desired and a piece of foil (0.8 × 2.5 cm.) was fitted to each, so that the seam would fall outside. A close fit was obtained by slitting the lower edge of the foil so that it could be folded around the end of the tip; excess foil was then carefully trimmed away. Spot-welding to form a practically continuous seam completed the assembly. The welding operation is simple with the proper equipment. In this case, a low inertia bench welder with synchronous thyratron control was used under the following conditions: 350 amperes for 6 cycles, or 0.1 second; electrode pressure, 20 pounds; flat copper electrodes of diameters $^3/_{16}$ inch (lower) and $^3/_{32}$ inch (upper).

The forceps thus constructed have been used constantly during the last six months and have given satisfactory service without deteriorating noticeably. There is no reason why the tips of a wide variety of tongs and forceps could not be covered with platinum foil to great advantage. The implements to be covered should preferably be made of stainless alloys.

A Large-Scale Laboratory Extractor of the Soxhlet Type

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IN CHEMICAL investigations of agricultural products it is frequently necessary to extract large quantities of material. This problem was encountered by the authors in an investigation on tobacco and its solution may be of use in other laboratories.

The extractor was designed and constructed from two alcohol drums and other materials obtained from a plumbing shop.

In Figure 1 the distilling pot, 1, is made from a 55-gallon drum. Heat is supplied by a steam coil entering at valve 2. The solvent vapor tube, 3, made of 2-inch iron pipe and fittings, is welded to the drums. A 30-gallon drum comprises the extraction chamber, 4, with 0.5-inch pipe and fittings for the siphon tube return, 5. Coupling joints are provided for both vapor tube and siphon tube for ease in handling. Condensation is maintained by a spiral cone condenser head, 6, with auxiliary straight tube condenser, 7, connected in series to the water line. A steam-radiator safety valve, 8, is attached to 7 when the air has been forced from the system. This prevents air from being drawn in by the partial vacuum created each time the extract siphons over, resulting in the loss of solvent.

A detailed diagram of the condenser-head assembly is shown in Figure 2. The drumheads are cut out and flanges welded to the sides. The extractor heads are bolted to these flanges, being sealed with braided graphite-impregnated packing. Thus they are removable for charging and cleaning. An ordinary 18-inch boiler gage, 9, provides a means for following the solvent level in the pot. The pot steam coil is so placed that the solvent level never falls below about 5 gallons, automatically preventing the extract from becoming overheated.

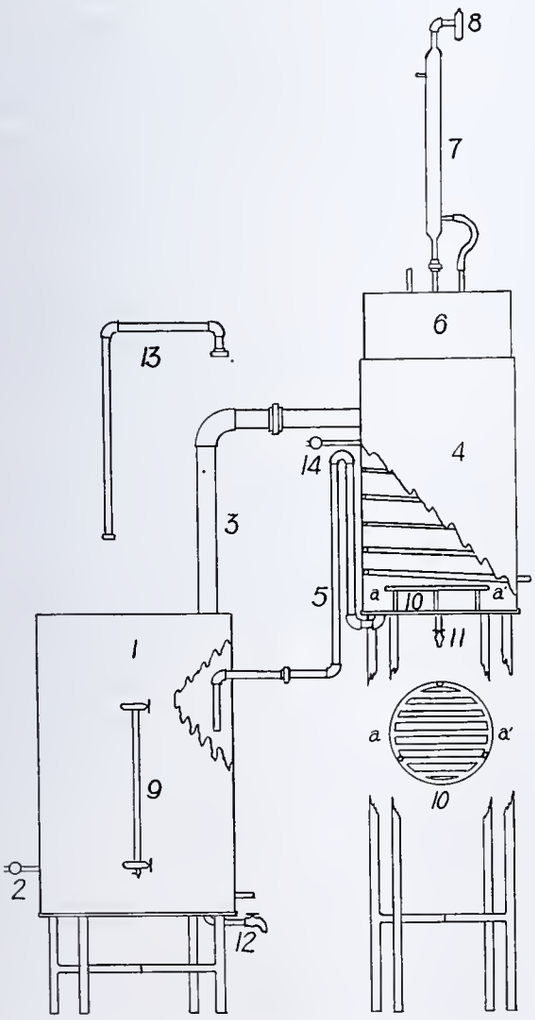

FIGURE 1. DIAGRAM OF APPARATUS

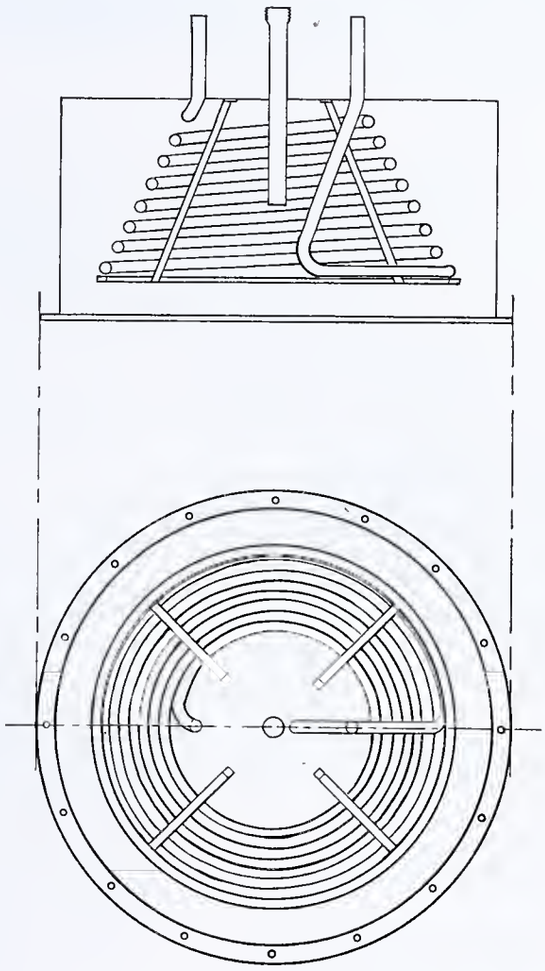

FIGURE 2. CONDENSER-HEAD ASSEMBLY

The ground material to be extracted is held in a heavy cotton bag double-sewn at the seams, which is supported on a small rack, 10, made of iron piping welded at the joints.

When extraction is complete the distilled solvent and concentrated extract solution are drawn off at taps 11 and 12, respectively. Following this operation the straight tube condenser, 7, is connected for distillation by means of adapter 13, the spiral cone condenser being disconnected from the water line. Steam through valve 14 is then used to distill the solvent from the extracted residue in the bag. Solvent vapor passes to the condenser and also in reverse direction through the vapor tube, 3, and is condensed. That returning to the distilling pot serves to rinse down the sides of the drum. It is drawn off and added to the concentrated extract for final solvent recovery.

The capacity of this apparatus is approximately 50 pounds of dried plant material. With this charge the solvent requirements are 25 to 35 gallons, most of which is recovered after extraction.

Experiments in the last year have proved the efficiency of the extractor.

THE investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the Director

Direct Quantitative Determination of Nicotinamide in Vitamin Mixtures

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With the approval and cooperation of the authors concerned, the ANALYTICAL EDITION presents here the results of research conducted independently in two laboratories on the same problem (see page 355). Using the same fundamental procedure with minor modifications in analytical technique, the investigators acquired data which offer confirmatory evidence on the value of their method.

That credit for priority in this work may be established, it should be noted that Dr. Lamb's method was written up in preliminary form last November and submitted to this journal on March 2. The method by Drs. Melnick and Oser was written up in February this year, and a manuscript submitted for publication on March 11.

THE method for the quantitative determination of nicotinic acid and nicotinamide based on the color reaction of König (*3*) has been employed successfully by a number of workers (*4-7*) in the analysis of both biological and vitamin concentrate materials. A discussion of the chemistry involved in this determination as well as references to numerous modifications and variations of the method is given in a review by Waisman and Elvehjem (*9*).

However, as with other physicochemical methods for the determination of nicotinic acid, the method is not specific, since other pyridine compounds also produce the same color upon treatment with cyanogen bromide and an aromatic amine. For this reason the usual practice is to hydrolyze the sample with either strong acid or alkali to convert other nicotinic acid derivatives to the free nicotinic acid. In this connection Melnick, Robinson, and Field (*6*) have reported that in the analysis of urine it is possible to obtain a semiquantitative analysis of the amounts of nicotinic acid, nicotinamide, nicotinuric acid, and trigonelline present by determining the apparent "nicotinic acid" values after various hydrolysis treatments.

Since today approximately 90 per cent of the compounded synthetic vitamin mixtures contain nicotinamide rather than nicotinic acid, it is highly desirable to have a direct quantitative method for the analysis of vitamin mixtures for nicotinamide. The microbiological method is not satisfactory for this purpose, since equimolecular weights of nicotinic acid, nicotinamide, and nicotinuric acid have equal activities for the organism used (*8*). In the case of the colorimetric method Melnick and Field (*5*) stated that the amide reacted with cyanogen bromide and aniline to give the same yellow color but the intensity of the color produced was about half that produced by an equal amount of nicotinic acid. Shaw and Macdonald (*7*) reported that when using nicotinamide the results were neither constant nor reproducible, and were generally lower than those obtained with the acid, while Bandier and Hald (*1*), using Metol as the aromatic amine, stated that the yellow color produced with the amide was usually considerably stronger than that obtained with the acid, and that the results were not reproducible.

Time-reaction studies have been made of the intensity of the color produced by treating both nicotinic acid and nicotinamide with aniline and cyanogen bromide.

For these studies equal quantities of acid and amide were used. Briefly, to 50 micrograms of the acid or amide in 12 ml. of distilled water, 1 ml. of 4 per cent aniline in 95 per cent ethanol was added, followed by 6 ml. of cyanogen bromide reagent (approximately 4 per cent). The solutions were quickly mixed, stirred to free them from air bubbles, and placed in specially matched tubes. Using the Evelyn photoelectric colorimeter with a 420 mμ filter, the intensity of the color produced was measured every 15 seconds.

The results of a typical set of these measurements are shown graphically in Figure 1, in which corrected extinction ($\log I_0/I$) values are plotted against time elapsed after addition of the cyanogen bromide reagent. A number of similar series of measurements have established that the time-reaction curves for nicotinic acid and nicotinamide are equally characteristic and reproducible. However, it is evident that the maximum intensity is reached in less time and decreases much faster for the amide than for the acid form. These curves also show that determinations based upon maximum readings are more reliable than determinations based upon readings taken at an arbitrary elapsed time of 5 minutes, since the readings

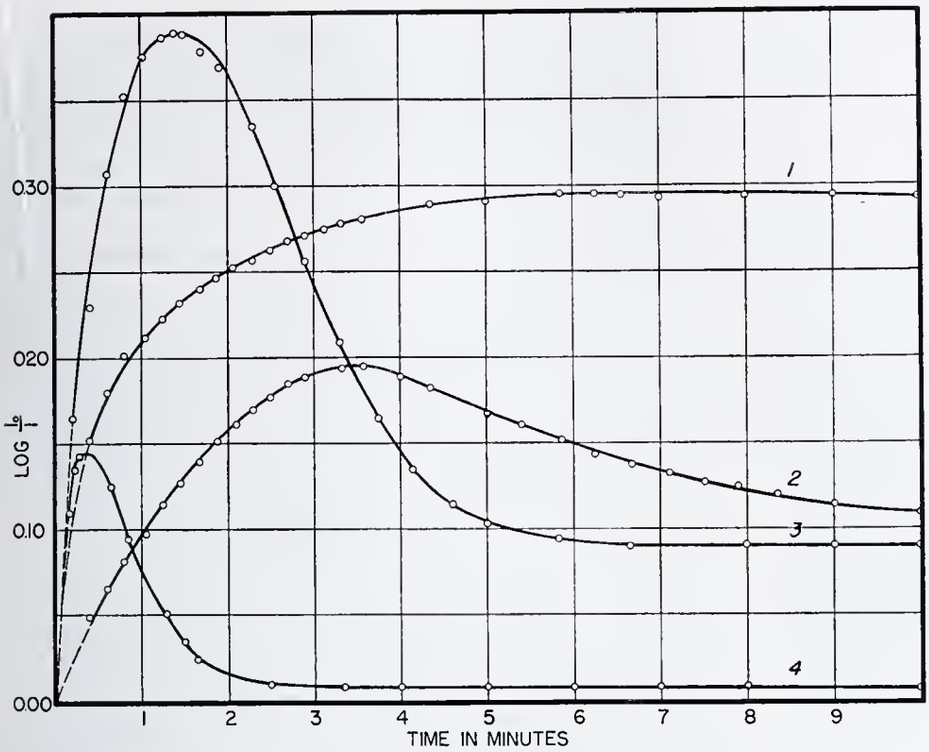

FIGURE 1. TIME-REACTION CURVES
50 micrograms of nicotinic acid (1), nicotinamide (2), pyridine (3), and β-picoline (4)

might vary considerably for a small difference in timing, particularly in the case of the amide.

The length of time for the maximum depth of color to develop varies somewhat with different cyanogen bromide preparations, and the rate of reaction increases with temperature. Various solvents have been found to change the rate of reaction—methyl alcohol, for example, retards the reaction considerably, the maximum color intensity for the amide being reached at the end of 6 to 7 minutes instead of 3 to 3.5 minutes. However, for normal laboratory conditions, maximum readings obtained in the analysis of standard nicotinic acid and nicotinamide solutions repeated from time to time during an 8-hour period were found to be reproducible to within ±2 per cent. In Figure 2 the maximum extinction coefficients for both nicotinic acid and nicotinamide as obtained by the above-described procedure are plotted against concentrations of these compounds in micrograms. This relationship was found to be linear for 0 to 75 micrograms of nicotinamide and for 0 to 100 micrograms of nicotinic acid. Since Beer's law fails to hold above these amounts, it is necessary to select sample aliquots so that the quantities of nicotinic acid and nicotinamide will fall within these ranges of concentration.

These observations form the basis of a method for the direct quantitative determination of nicotinamide which has been used successfully in the analysis of several hundred samples of synthetic vitamin mixtures. As with other chemical methods, this method is also open to criticism in that it is not entirely specific for either form. However, by making time-reaction studies it is possible to detect very quickly whether a material contains nicotinic acid, nicotinamide, or a mixture of the two, since the relationship of the extinctions of the resulting colors to time of their development is distinctly different for the acid and amide. Having determined the qualitative composition of the sample in respect to these substances, the material may then be analyzed for either nicotinic acid, its amide, or both, as described under procedure for analysis. If the time-reaction curve indicates that the sample contains a mixture of the amide and acid, it is necessary to make a time-reaction curve both before and after hydrolysis of the sample. Using Equation 1, it is then possible to calculate the amounts of amide and acid present. While the preparation of reagents, general procedure for analysis, and treatment of results are given in the literature, it is thought worth while to repeat these briefly with the inclusion of a few helpful details gained from experience in using this method during the past year.

Experimental Procedure

PREPARATION OF REAGENTS. The cyanogen bromide reagent prepared in 4-liter quantities as described below has been found to be stable when stored at room temperatures for 2 months or more. A saturated bromine solution is prepared by dissolving 57 ml. of bromine in 4 liters of distilled water at 5° to 10° C. This solution is stirred continually while a 10 per cent sodium cyanide or potassium cyanide solution is carefully added until completely decolorized. An excess of cyanide is to be avoided. As soon as the solution becomes a pale green-yellow its per cent transmission is measured after each small addition of the cyanide solution. When the per cent transmission of the cyanogen bromide solution reaches a maximum, sufficient cyanide solution has been added. The amount of 10 per cent potassium cyanide required to decolorize 4 liters of the saturated bromine water is roughly 725 ml. The resulting 4 to 5 per cent cyanogen bromide solution is conveniently stored in a 2-liter automatic buret, from which it may be easily and safely dispensed.

A 4 per cent aniline solution is prepared from C. P. aniline in 95 per cent ethanol and stored in an amber bottle.

Standard solutions of nicotinic acid and nicotinamide are prepared containing 25 micrograms per ml. in distilled water.

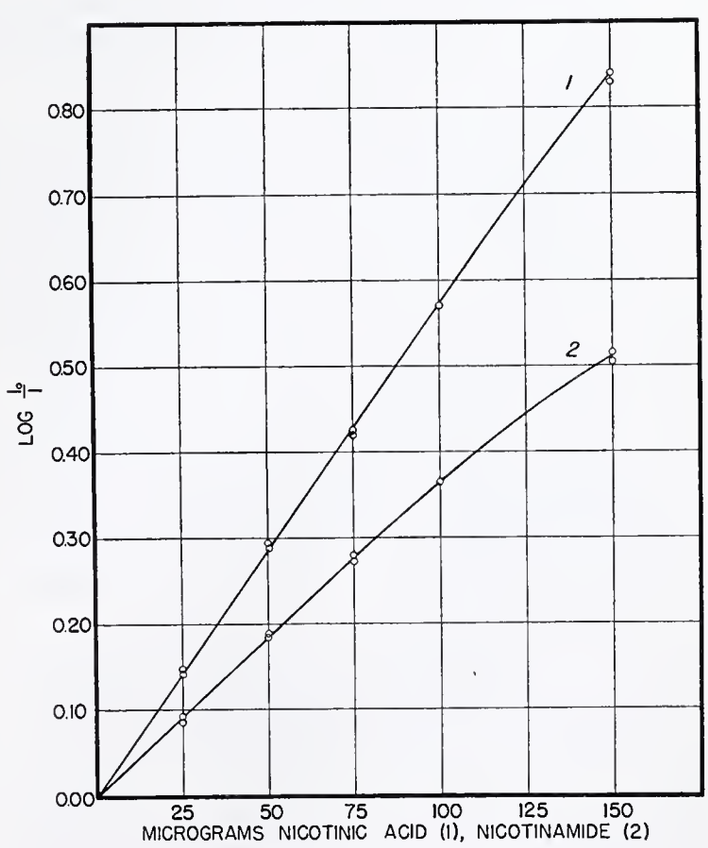

FIGURE 2

Table I. Effect of Time Delay in Adding Cyanogen Bromide Following Addition of Aniline

Time[a]	Log $\frac{I_0}{I}$ for 50 μg. of Nicotinamide	Change in Log $\frac{I_0}{I}$	Log $\frac{I_0}{I}$ for 50 μg. of Nicotinic Acid	Change in Log $\frac{I_0}{I}$
Min.		%		%
0	0.195	0.00	0.295	0.00
2	0.189	−3.08	0.300	+1.69
4	0.192	−1.54	0.298	+1.02
8	0.196	+0.51	0.297	+0.68
16	0.196	+0.51	0.305	+3.39

[a] Elapsed time between addition of aniline and cyanogen bromide.

These standard solutions have been found to be stable as long as 2 weeks; however, it is advisable to prepare fresh solutions each week.

Preparation of Sample. Aqueous solutions of samples are prepared to contain 1 to 30 micrograms of nicotinic acid or amide per ml. In the case of synthetic vitamin mixtures, tablets, and capsules it is convenient to place the required number in a volumetric flask, heat on a steam bath, and swirl until the material is either completely in solution or uniformly dispersed. The addition of 1 ml. of ethylene dichloride aids by quickly dispersing vitamin mixtures containing waxes, fats, oils, liver concentrates, etc. The solutions are then made to volume and filtered to remove oils, fats, waxes, and other insoluble material. The first 50 to 100 ml. of filtrate are discarded to avoid loss of nicotinic acid and amide by adsorption on the filter paper. While it may not be accepted quantitative procedure, it has been found helpful to add a drop or two of a 2 per cent aerosol solution to the filtrate to aid in more accurate pipetting of the sample aliquots. Iron present in appreciable amounts forms a troublesome greenish-blue complex with cyanide. This may be avoided by treating the warm solutions with potassium hydrogen phosphate, the precipitated iron phosphate being removed in the subsequent filtration.

Treatment with Cyanogen Bromide and Aniline. Six 25-ml. glass-stoppered graduates are labeled 1, 2, 3, 4, 5, and 6. Graduates 1 and 2 are reagent blanks and need to be repeated only once or twice daily, 3 is a solution blank, 4 is the sample, and 5 and 6 are the sample to which known amounts of nicotinic acid or amide are added. If the sample contains nicotinic acid, known amounts of nicotinic acid are added; if the sample contains the amide, known amounts of nicotinamide are added. To graduates 1 and 2 are added 12 ml. of distilled water. Into 3, 4, 5, and 6 is pipetted 1 ml. of the sample solution containing 10 to 30 micrograms of acid or amide per ml. If the sample solution contains 1 to 5 micrograms per ml., 10 ml. of the sample solution are used; if 5 to 10 micrograms, 5 ml. are used. Eighteen milliliters of distilled water are added to 3, and distilled water is added to 4, 5, and 6 to make a total volume of 12, 11.5, and 11 ml., respectively. One-half milliliter of the standard solution (acid or amide) is added to 5, and 1.0 ml. of the respective standard solution to 6. Next, 1 ml. of 4 per cent aniline solution is added to each of the six graduates, followed by 6 ml. of the cyanogen bromide reagent.

It is important to add the aniline first, since, when cyanogen bromide is added first, the longer the delay before adding the aniline the more intense is the maximum color developed. Adding the aniline first, the elapsed time before adding the cyanogen bromide is not critical, as is shown in Table I, where the per cent differences for individual determinations are within the limits of error of the method—namely, ±2 per cent. Table II indicates that in the case of the amide as great as a 50 per cent increase in log I_0/I may be introduced by adding the cyanogen bromide first and the aniline 10 minutes later, while a delay of 8 minutes introduces an increase of 12 per cent in the case of nicotinic acid. Advantage has been taken of this increase in photometric density in the collaborative method described by Melnick (*4*) for the determination of nicotinic acid in cereal products. In this method the cyanogen bromide is added first, followed in exactly 10 minutes by the addition of the aniline reagent. The data in Table II, column 5, show the importance of exact timing when this procedure is followed.

As soon as cyanogen bromide reagent is added, the time is noted and the solutions are well mixed and immediately placed in specially matched test tubes. The change in per cent transmission with time is measured on an Evelyn photoelectric colorimeter with a 420 mμ filter. Solutions 1 and 2 are read against water as a blank—i. e., the galvanometer is adjusted to 100 per cent transmission with distilled water. Solution 3 is used to adjust the galvanometer to 100 per cent transmission before measuring solutions 4, 5, and 6 in order to compensate for the color of the original sample solution. In the case of an unknown sample, it is necessary to make a complete series of transmission measurements from 15 seconds to 8 minutes in order to ascertain the nature of the sample. However, in the case of samples with known history it is only necessary to have each solution in the instrument in time to take readings before the maximum is reached, in order to be certain that a measurement is made at the maximum intensity. This is particularly important in the case of the amide, as is seen in Figure 1.

Treatment of Results. In using an instrument such as the Evelyn photoelectric colorimeter, the measurements are made as per cent transmission which are converted to extinction coefficients ($\log \frac{I_0}{I}$). The average value of the extinction coefficients of the reagent blanks (solutions 1 and 2) is then subtracted from the extinction coefficients of sample solutions 4, 5, and 6. These corrected extinction coefficients are plotted as ordinates against the added amounts of nicotinic acid or amide as abscissas. Since it has been found that Beer's law holds for these concentrations, the three points at 0, 12.5, and 25 micrograms of added nicotinamide or acid will determine a straight line. Extrapolation of this straight line through the abscissa gives the amount of nicotinic acid or nicotinamide, as the case may be, originally present in the sample aliquot. By careful technique results may be checked to within ±2 per cent. As stated by Harris and Raymond (*2*), the advantage of treating results in this manner lies in the fact that while the slope of the line may vary somewhat with different samples because of variations in pH, salt concentration, etc., yet the three points will still determine a straight line from which an accurate determination may be made.

Table II. Effect of Time Delay in Adding Aniline Following Addition of Cyanogen Bromide

Time[a]	Log $\frac{I_0}{I}$ for 50 μg. of Nicotinamide	Change in Log $\frac{I_0}{I}$	Log $\frac{I_0}{I}$ for 50 μg. of Nicotinic Acid	Change in Log $\frac{I_0}{I}$
Min.		%		%
0	0.195	0.00	0.295	0.00
1	0.207	+ 6.02	0.308	+ 4.38
2	0.222	+14.03	0.308	+ 4.38
4	0.238	+22.33	0.315	+ 6.64
5	0.246	+26.11	...	...
8	...	...	0.333	+12.82
10	0.298	+52.62	...	...
16	...	...	0.364	+23.45

[a] Elapsed time between addition of cyanogen bromide and aniline.

In the analysis of samples containing both nicotinamide and nicotinic acid, the samples are first run directly as the amide and then following hydrolysis determined as total nicotinic acid. By use of Equation 1 the amounts of nicotinic acid and nicotinamide may be determined.

Let T = total micrograms of nicotinic acid plus nicotinamide as determined on an aliquot of the hydrolyzed sample
x = micrograms of nicotinamide originally present in an equal sample aliquot
y = micrograms of nicotinic acid originally present in an equal sample aliquot
z = micrograms determined as amide before hydrolysis on an equal sample aliquot
K = ratio of the maximum extinction of the acid to that of an equal quantity of amide at time of maximum amide intensity as obtained from time-reaction curves of standard nicotinic acid and nicotinamide solution similar to Figure 1

Since the micrograms determined as amide before hydrolysis are equal to the actual micrograms of nicotinamide plus the micrograms of nicotinic acid times the factor K, the following equation may be written:

$$z = T - y + Ky$$

$$y = \frac{T - z}{1 - K} \qquad (1)$$

from which y may be determined, giving the micrograms of nicotinic acid, while y subtracted from T gives the micrograms of nicotinamide present in the sample aliquot.

Small experimental errors in the determination of z or T or both become magnified in solving for x and y. As a result, individual determinations for nicotinic acid and nicotinamide in materials containing both forms may differ from one another by as much as 10 or 15 per cent.

Table III. Extinctions of Equimolecular Quantities

Compound	Equimolecular Weights	$\text{Log}\frac{I_0}{I}$	Time[a]
	μg.		Sec.
Nicotinic acid	50.0	0.295	360–420
Nicotinamide	49.6	0.195	210
Pyridine	32.0	0.248	87
β-Picoline	38.0	0.111	28

[a] Length of time for development of maximum extinction.

Discussion

In Figure 1 time-reaction curves are given for β-picoline and pyridine as well as for nicotinic acid and nicotinamide. A comparison of these curves shows that the more polar the group on the beta-carbon atom of the pyridine nucleus the longer the time required for maximum development of color and the more stable the color produced. These results indicate that the nature of the group on the beta-carbon atom of the pyridine nucleus exerts a decided influence on the mechanism of the dianil reaction and on the intensity of the color of the resulting compound. In Figure 1 maximum extinction coefficients are plotted for equal quantities of compounds—namely, 50 micrograms. Maximum extinction coefficients for equimolecular quantities of the four compounds have been calculated from Figure 1 and tabulated in Table III. On an equimolecular basis the intensity of the color produced by these four compounds decreases in the following order: nicotinic acid, nicotinamide, pyridine, and β-picoline. This is the same order as reported by Melnick, Robinson, and Field (*6*).

Summary

A method for the direct quantitative analysis of compounded vitamin mixtures for nicotinamide has been described, which has been used successfully in the analysis of several hundred samples during the past year. Time-reaction measurements of the color produced by the König reaction, using the reagents cyanogen bromide and aniline, form the basis of the method. The relationship between maximum extinction coefficients and time for their development has been found to be distinctly characteristic for nicotinic acid and nicotinamide as well as for pyridine and β-picoline.

A delay of a few minutes in the addition of aniline following the addition of cyanogen bromide introduces considerable error, while as much as a 15-minute delay in adding cyanogen bromide after first adding the aniline does not introduce an appreciable error.

Results obtained by this method have been found to be reproducible to within ±2 per cent for the direct quantitative determination of either nicotinic acid or nicotinamide in vitamin mixtures.

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Chemical Differentiation between Niacinamide and Niacin in Pharmaceutical Products[1]

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THE microbiological method of Snell and Wright in its existing forms (*1*, *2*, *5*) does not distinguish between niacin and niacinamide, since both compounds exert the same microbiological stimulatory effects (*5*). By taking advantage of the relative reaction rates, the chemical method offers a means of differentiating between the free acid and the amide. This distinction is of no little importance, since niacin produces in many individuals an uncomfortable sensation of flushing and erythema of the skin; moreover, the amide is more expensive. In the chemical method (*2*, *3*) the samples are first subjected to acid hydrolysis to convert biologically active niacin derivatives to free niacin. Niacinamide, however, reacts with the reagents prior to hydrolysis to yield the same color as niacin but of lesser intensity (*3*). Niacinamide, like niacin, is quantitatively adsorbed on and eluted from Lloyd's reagent and neither is lost during the lead hydroxide clarification procedure (*2*). The color reactions due to these two compounds in one solution are additive.

When the color reaction is carried out according to the collaborative chemical procedure (*2*), the maximal color developed (the photometric density 3 to 8 minutes after the addition of the aniline reagent) is 35 to 40 per cent greater with niacin than with an equivalent amount of niacinamide. With the latter, the maximal photometric density develops in approximately 2.5 minutes but is stable for only 30 seconds. If the cyanogen bromide and aniline reagents are added in rapid sequence as in the method of Melnick and Field (*3*)—i. e., omitting the 10-minute period during which the cyanogen bromide alone is allowed to react—the sensitivity of the method for niacin is reduced by 30 to 35 per cent, but the maximal color due to reacted niacin is now practically twice that of reacted niacinamide. Figure 1 shows these differences in reaction rates. Readings were taken at 15-second intervals

[1] See Editor's note on page 352.

in an Evelyn photoelectric colorimeter (Rubicon Company, Philadelphia, Penna.) with filter 420.

In conducting a test for differentiating niacin from niacinamide, the material is tested before and after acid hydrolysis. The molecular weights of niacin and niacinamide are almost the same, 123 and 122, respectively. The details for the preparation of the reagents, the hydrolysis, subsequent clarification, and the setting up of the solutions for the color development have been described in detail elsewhere (*2*). The final color development, however, follows the earlier method of Melnick and Field (*3*). [For the first test of total niacin content the collaborative chemical procedure (*2*) may be used. However, for testing the unhydrolyzed sample the addition of the reagents in rapid sequence (*3*) is preferred, in order that the photometric densities of reacted niacin and niacinamide may show a bigger difference. The readings should be taken periodically during the color development, since maximal constant values are required for the calculation and the reaction, particularly with niacinamide, fades rapidly once the maximum is attained.]

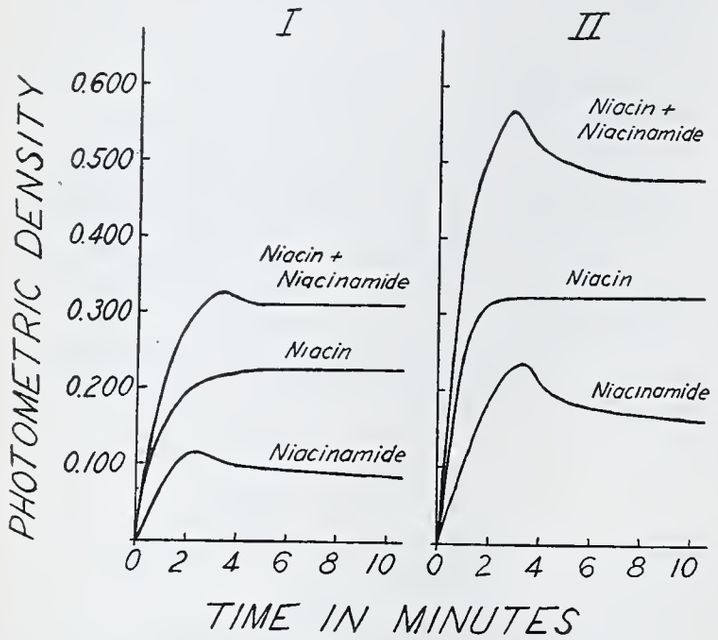

FIGURE 1. TIME-REACTION CURVES

10-microgram quantities of niacin and niacinamide with cyanogen bromide and aniline reagents.
I. Reagents added in rapid sequence (*3*)
II. Cyanogen bromide allowed to react with vitamin for 10 minutes before addition of aniline reagent (*2*)

Typical Calculation

Test substance: vitamin capsule subjected to acid hydrolysis.

Preparation of test solution: 20 capsules dissolved in 200 ml. of water; 1-ml. aliquot carried through the collaborative chemical method (*2*) but the maximal yellow color due to reacted niacin measured after the cyanogen bromide and aniline reagents were added in rapid sequence (*3*).

Eight milliliters of the final neutralized supernatant solution are diluted to 10 ml. and 3-ml. aliquots taken for testing.

0.016 = photometric density of the blank

0.438 = photometric density of the test solution

0.699 = photometric density of the test solution containing 10 μg. of added niacin

Then

0.438 − 0.016 = 0.422 photometric density due to reacted niacin

0.699 − 0.438 = 0.261 photometric density due to 10 μg. of reacted niacin

0.422/0.261 × 10 = 16.2 μg. of niacin in 3-ml. aliquot of test solution

16.2 × 10/3 × 15/8 × 1000/1 × 1/10 × 1/1000 = 10.1 mg. of total niacin per capsule

Test for niacinamide: repeat above, omitting acid hydrolysis

0.000 = photometric density of the blank

0.216 = photometric density of test solution

0.475 = photometric density of test solution containing 10 μg. of added niacin

0.216 − 0.000 = 0.216 photometric density due to reacted niacin compounds

0.475 − 0.216 = 0.259 photometric density due to 10 μg. of added niacin

0.216/0.259 × 10 = 8.3 μg. of apparent niacin in sample

8.3 × 10/3 × 15/8 × 1000/1 × 1/10 × 1/1000 = 5.2 mg. of apparent niacin per capsule

Since this is one half of the total niacin, found after hydrolysis, the material is present in the capsule entirely as niacinamide.

Had the above value for apparent niacin been 7.0 mg. per capsule, then

$$\begin{array}{r} 10.1 - 7.0 = 3.1 \\ 2 \\ \hline 6.2 \end{array}$$ mg. of niacinamide per capsule

and 10.1 − 6.2 = 3.9 mg. of niacin per capsule

Some latitude (±15 per cent) should be allowed in interpreting the results, since the accuracy of the partition of the niacin ingredient is dependent upon the precision of two assays and errors may be additive.

In testing samples such as pure niacin, niacinamide, or mixtures of the two, clear colorless solutions are obtained; the adsorption-elution and decolorization procedures are then omitted. Greater precision is also obtained. For determinations conducted on pure niacin or niacinamide preparations, direct reference to the reaction curves in Figure 1 may suffice to identify and allow rapid quantitative estimation of the compound with a good degree of precision (±2 per cent).

The nicotinamide-containing coenzymes fail to yield a yellow reaction product with the reagents unless first hydrolyzed. Nicotinuric acid reacts with the reagents to yield some color prior to hydrolysis. Strong acid hydrolysis is required to convert it quantitatively to free niacin (*4*). However, these compounds are not readily available. The test procedure described is intended for use only in assaying the niacin ingredient in vitamin capsules and tablets containing the synthetic vitamin.

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Determination of Microquantities of Certain Proteins

A Colorimetic Method

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IN CONNECTION with studies of serological reactions (*5*) it was necessary to have a simple and rapid method for determining quantitatively small amounts (from 10 to 1000 micrograms) of specifically precipitated antibody globulin which would permit a large number of determinations in a short time. A colorimetric method using the Folin-Ciocaltéu phenol reagent (*3*) was finally chosen. The modification of heating the protein in alkaline solution before the addition of the phenol reagent to increase the intensity of the color (*1*, *4*) was also adopted.

The phenol reagent reacts with the chromogenic reducing groups of proteins in basic solution to give a blue color. However, the phenol reagent is decomposed rapidly by alkali, and thus the color developed depends on the relative rates of reaction of the phenol reagent with protein and with alkali. In most cases the protein does not react completely, and this cannot be remedied by use of larger amounts of the reagents, since then white precipitates form rapidly. Therefore, an empirical procedure which would give reproducible results was developed, and the effect of pertinent variations was studied.

Procedure

Add from a buret 2.5 ml. of 1.00 *N* sodium hydroxide solution to 10 to 1000 μg. of the protein sample in a calibrated 15-ml. centrifuge tube and bring to a volume of 7.5 ml. with distilled water. Specific precipitates dissolve readily in this solution. Heat in a boiling water bath from 5 to 10 minutes and cool to about 25° C. in running tap water. Add rapidly 2.5 ml. of the phenol reagent (prepared as described by Folin and Ciocaltéu, *3*, and diluted with two volumes of water) and shake well immediately. A clean rubber stopper may be used during the shaking without affecting the color. Let stand 10 minutes for color development and read the color intensity with a Klett-Summerson photoelectric colorimeter, using a red filter transmitting 640 to 700 mμ. If the reading is above 450 units, dilute the solution two- or fourfold and multiply the observed reading by the dilution factor. Make a blank run at the same time, omitting the protein, and subtract the blank reading (10 to 15 units) from the protein color reading. Read the amount of protein present from a plot, such as that in Figure 1, of the corrected color reading against the weight of protein.

The author has found it convenient to run the determinations in sets of twelve. By staggering the sets, one man can average 40 analyses per hour or two men can average 100 analyses per hour with a single instrument.

Using simple substances as antigens (*5*), the author found that the antigen usually has a negligible chromogenic value. When colored proteins are used as antigens, the amount of antigen in the precipitate can be determined colorimetrically before the addition of the phenol reagent and a correction can be made for the chromogenic value of the antigen.

Although the same amounts of different proteins have different chromogenic values, the plots are similar. By changing the protein scale (abscissa), the plots can be made the same for rabbit globulin, bovine globulin, crystalline ovalbumin, and whole rabbit serum.

The plot of Figure 1 was made for rabbit serum globulin (rabbit anticrystalline ovalbumin) by the following procedure:

A solution of ovalbumin of known concentration as determined by Kjeldahl analysis (*6*) was added to an excess of rabbit anti-ovalbumin serum and the mixture was permitted to stand overnight in the refrigerator. The precipitate (*ca.* 12 mg.) was washed three times with 20-ml. portions of 0.9 per cent saline, dissolved in 1.00 *N* sodium hydroxide solution, and made up to a definite volume as a stock solution. Aliquots of this solution were analyzed by the Kjeldahl method for nitrogen and the amount of protein was calculated by multiplying the weight of nitrogen by the factor 6.25. Dilutions of the stock protein solution were made and analyzed colorimetrically by the method outlined above. The amounts of protein and the color readings were corrected for the small amount of ovalbumin present. All the ovalbumin added was assumed to be in the precipitate, since the precipitate was obtained in the region of antibody excess. The experimental points used in Figure 1 were run in duplicate with average deviation of ±1.5 per cent.

The phenol reagent was checked from time to time against a standard solution of tyrosine in hydrochloric acid stabilized with formalin, as described by Anson (*2*), and was found to give the same color intensity over a period of 4 months and for three different preparations of the reagent. This indicates the stability and the reproducibility of the reagent.

In order to check the effect of variations from the chosen procedure, experiments were carried out to study the effect of varying the amount of sodium hydroxide used; the amount of phenol reagent used; the length of time of exposure to sodium hydroxide before, during, and after heating; the

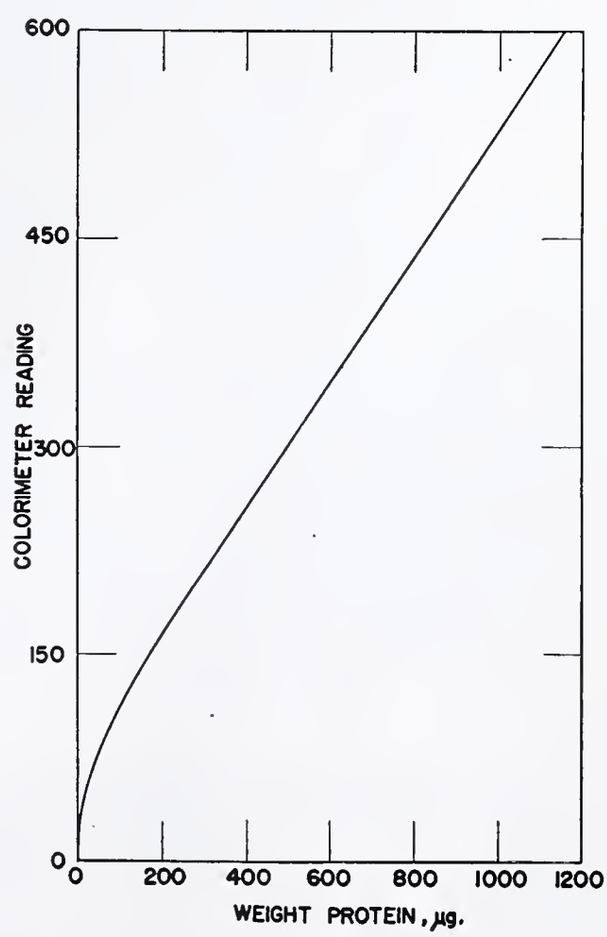

FIGURE 1. PLOT OF COLORIMETER READING AGAINST WEIGHT OF RABBIT ANTIBODY GLOBULIN

Table I. Effect of Concentration of Reagents

(4 ml. of stock globulin solution)

NaOH	Phenol Reagent	Color[a] Reading
Ml.	*Ml.*	
2.5	1.8	305
2.5	1.9	300
2.5	2.0	323
2.5	2.1	340
2.5	2.2	350
2.5	2.3	355
2.5	2.4	370
2.5	2.5	398
2.5	2.6	378
2.5	2.7	380
2.5	2.8	365
2.5	2.9	320
2.5	3.0	285
2.5	3.1	240
2.5	3.2	234
1.8	2.5	188
1.9	2.5	218
2.0	2.5	242
2.1	2.5	308
2.2	2.5	355
2.3	2.5	390
2.4	2.5	397
2.5	2.5	400
2.6	2.5	390
2.7	2.5	373
2.8	2.5	358
2.9	2.5	350
3.0	2.5	345
3.1	2.5	330
3.2	2.5	327
1.0	1.0	197[b]
2.0	2.0	225[b]
3.0	3.0	244[b]
4.0	4.0	Ppt. formed[b]

[a] Average of two determinations with average deviation of 2.0%.
[b] One-half amount of protein used.

length of time of reaction with the phenol reagent; the temperature of the reactants; and the mode of addition of the phenol reagent. The effect of omitting the heating step was also studied. The experiments were carried out with a stock solution of bovine globulin and the volumes and time intervals used were the same as those in the chosen procedure except for the specific variation studied.

Effect of Variations

The effect of the concentration of the reagents was determined by varying each while the other was kept constant (Table I). It is evident that the maximum color develops with equal volumes of the phenol reagent and sodium hydroxide, and that a slight error (less than 0.05 ml.) in the amount of either of the reagents added would have very little effect on the reading. The last four determinations in Table I indicate that the depth of color increases with increasing amounts of both reagents but that at high concentrations a white precipitate forms too rapidly. This does not take place nearly so rapidly with lower concentrations. That increased amounts of reagents give increased readings shows that the protein has not reacted as completely as is possible.

The effect of time of exposure to sodium hydroxide before, during, and after heating is shown in Table II. The protein solution can stand with the sodium hydroxide at least 4 hours before heating or 5 hours after heating without changing the final color value. The heating may continue from 3 to 14 minutes.

The concentration of sodium hydroxide during the 10-minute heating period is unimportant, since heating 5 ml. of protein solution with 0.5, 1.0, 1.5, 2.0, or 2.5 ml. of sodium hydroxide solution, followed by the subsequent addition of the remainder of the sodium hydroxide solution after cooling, gives identical final color readings.

The color value should not be read for at least 10 minutes after the addition of the phenol reagent, as is shown in Table III. The reading is then constant until a white precipitate begins to form. The time of formation of this precipitate is variable. It probably is a supersaturation phenomenon, since traces of the precipitate greatly increase the rate of precipitate formation.

The temperature at which the mixing takes place was varied (Table IV). Apparently the optimum temperature is room temperature, 20° to 25° C. The rate of addition of the phenol reagent is important, since adding the reagent with stirring in small portions over a period of 1 minute increased the color intensity by over 10 per cent. However, adding the reagent rapidly, all in one portion, followed by shaking immediately or even 1 minute later gave constant results.

If the heating step is omitted, the optimum volume ratio of phenol reagent to sodium hydroxide solution remains the same and the rate of color formation is similar to that when the solution is heated, but the maximum color is 20 per cent less. The main difficulty incurred when the heating is omitted is

Table II. Effect of Exposure to Sodium Hydroxide before, during, and after Heating

(5 ml. of stock globulin solution)

Time before Heating	Time during Heating	Time after Heating	Color[a] Reading
Min.	*Min.*	*Min.*	
1	10	...	463
3	10	...	452
6	10	...	452
15	10	...	455
30	10	...	452
60	10	...	457
120	10	...	463
240	10	...	463
10	0	...	360
10	1	...	430
10	2	...	442
10	3	...	450
10	5	...	447
10	10	...	440
10	14	...	435
10	30	...	430
10	10	5	438
10	10	10	433
10	10	15	428
10	10	30	442
10	10	60	448
10	10	160	443
10	10	300	452

[a] Duplicate analysis with mean deviation ±1%.

Table III. Color Readings after Addition of Phenol Reagent

(5 ml. of stock globulin solution)

Time	Color[a] Reading
Min.	
1	327
2	365
4	410
6	432
8	445
10	457
12	467
14	472
16	477
18	472
20	Ppt. forms

[a] Average of duplicate analyses with mean deviation of 1%.

Table IV. Effect of Temperature of Mixing

(5 ml. of stock globulin solution)

Temperature of Mixing	Color[a] Reading
° *C.*	
15	422
20	445
25	450
30	430
35	412

[a] Average of duplicate analyses with mean deviation of 1%.

Table V. Effect of Standing with Sodium Hydroxide without Heating

(5 ml. of stock globulin solution)

Time of Exposure	Color[a] Reading
Min.	
1	353
3	365
5	365
10	378
15	390
30	405
60	423
120	435
1440	437

[a] Duplicate analyses with mean deviation of 1.5%.

that the color reading depends on the time of exposure to sodium hydroxide for any relatively short time, as shown in Table V. This would require that subsequent steps in the analyses be delayed inconveniently for at least 2 hours, since in the author's work the protein samples are precipitates which are often slowly soluble in alkali, and thus no standard short time could be chosen. Heating with alkali presumably denatures the protein, so that all chromogenic groups are exposed, and thus any slight denaturation in the handling or aging of the protein (*4*) can be neglected.

Acknowledgment

This investigation was carried out with the aid of a grant from The Rockefeller Foundation. The author wishes to acknowledge the aid of Mrs. E. Swingle and A. Grossberg in checking the method.

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Contribution from the Gates and Crellin Laboratories of Chemistry, California Institute of Technology, No. 926.

Microdetermination of Volatile Matter in Coal and Coal Products

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The need for microdetermination of volatile matter arose in this laboratory in connection with a study of the combustion of pulverized coal (*6*). Volatile matter had to be determined on small amounts of physically similar particles which had been separated by microscopic examination. Since only small amounts of products were available from the combustion studies, the standard A. S. T. M. (*1*) procedure for determining volatile matter was impractical.

The method decided upon consists, essentially, of carbonization of a 3-mg. sample in a stream of dry, oxygen-free nitrogen. The sample is contained in a platinum microboat placed in a quartz combustion tube. Similar tube methods for macrodetermination of volatile matter have been described in the literature (*2, 3, 4*). This micromethod offers the advantages of introduction of the boat into a cold part of the tube, carbonization at a high heating rate, and cooling in an oxygen-free atmosphere. Oxidation of the sample is thereby minimized. No attempt has been made to obtain agreement with the standard A. S. T. M. method. Statistical analysis of the results obtained by the micromethod on 43 samples showed that this procedure gives results as reproducible as those obtained by the A. S. T. M. method. The statistical analysis was similar to one made on macro results for volatile matter (*5*). The limited data available indicate that there is a linear correlation between the standard differences of duplicate analyses and the absolute level of volatile matter. The results obtained by the micromethod are somewhat higher, owing to a higher rate of heating. Similar results have been observed previously (*7*).

Apparatus

The apparatus consisted of a quartz combustion microtube with a side arm. Nitrogen was freed of oxygen by passage through an electrically heated tube containing metallic copper and then passed in turn through a concentrated sulfuric acid wash bottle and a large U-tube filled with Anhydrone, which was connected to the side arm of the combustion tube. The sulfuric acid wash bottle served as a bubble counter. The combustion tube was heated electrically by a cylindrical furnace 22.5 cm. long and 11.5 cm. in diameter. The temperature gradient along the furnace was determined by means of a Chromel-Alumel thermocouple. Readings were taken at 1.5-cm. intervals on both sides of the center of the furnace with the thermocouple inserted in the combustion tube, nitrogen flowing at 10 cc. per minute. The maximum temperature difference for a length of 6 cm. was 5° C.

To permit introduction of the boat in a cold part of the tube, the furnace was mounted on a steel track. This permitted it to slide laterally along the length of the combustion tube and be replaced accurately at a given place around the tube.

The boat was placed in the tube and pushed to a fixed spot by means of a marked glass rod. This position was at the center of the furnace when the furnace was moved into position. To determine the rate of heating of the sample as it was being carbonized, "blank" runs were made with the thermocouple junction placed in the same position occupied by the boat in a determination with nitrogen flowing at the standard rate. The temperature reached 865 ± 5° C. in one minute and 950 ± 5° C. between the sixth and the tenth minute. Temperature constancy was maintained by manual control using a Varitran voltage regulator.

Method

Three milligrams of finely ground sample were spread uniformly along the bottom of a platinum microboat, dried in a Pregl block at 115° C. for 15 minutes, and weighed on a microbalance. The boat was introduced into the combustion tube and pushed into position with a glass rod. Dry nitrogen was passed through the combustion tube for 15 minutes at 10 cc. per minute to displace the air. The furnace was then moved along the track to a fixed position so that the boat was now at the center position. At the end of 10 minutes, measured with a stop watch, the furnace was moved away from the boat. The boat was permitted to cool for 7 minutes, preliminary tests with a thermocouple having shown that the temperature dropped to

Table I. Microdetermination of Volatile Matter

Sample	Volatile Matter Dry basis %	Volatile Matter Dry basis, A. S. T. M. %	Sample	Volatile Matter Dry basis %	Volatile Matter Dry basis, A. S. T. M. %	Sample	Volatile Matter Dry basis %	Volatile Matter Dry basis, A. S. T. M. %	Sample	Volatile Matter Dry basis %	Volatile Matter Dry basis, A. S. T. M. %
Coke 1	1.02		Coal residue 5	10.17		Coal 1	17.34		Coal 6	38.15	
	1.13			9.91			17.49			38.35	
	1.17			Av. 10.04	..		17.61			Av. 38.25	..
	1.04		Coal residue 6	10.67			Av. 17.48	15.4	Coal 7	36.22	
	1.09			10.61		Coal 2	36.76			36.06	
	1.17			Av. 10.64	..		36.93			Av. 36.14	..
	Av. 1.10	0.99					36.60		Coal 8	30.15	
Coke 2	0.84						37.03			30.43	
	0.94						36.53			30.19	
	Av. 0.89	0.39					36.91			Av. 30.26	..
Coal residue 1	1.23						Av. 36.79	34.3	Coal 9	28.61	
	1.35					Coal 3	38.88			28.63	
	Av. 1.29	..					38.13			Av. 28.62	..
Coal residue 2	1.19						38.26		Coal 10	36.53	
	1.28						38.54			36.69	
	Av. 1.24	..					Av. 38.45	37.5		36.38	
Coal residue 3	1.60					Coal 4	38.29			Av. 36.53	..
	1.44						38.49		Coal resin	89.12	
	Av. 1.52	..					Av. 38.39	..		89.04	
Coal residue 4	2.77					Coal 5	38.22			Av. 89.08	..
	2.80						38.18				
	Av. 2.79	..					Av. 38.20	..			

40° C. in this period. The boat and residue were removed and placed on a cooling block for 5 minutes and weighed. In cases of low volatile samples more accurate results were obtained by weighing the boat and residue in a glass-stoppered pig. The results on various samples are indicated in Table I.

Summary

A micromethod has been described which has the same reproducibility as the A. S. T. M. macroprocedure, but gives somewhat higher results. Carbonization, sample introduction, and sample removal occur under conditions which minimize air oxidation. The method can be applied to samples varying over a wide range of volatile matter without modifying the technique.

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New Reactions of 2,2′-Dichlorodiethyl Sulfide

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THE reaction of 2,2′-dichlorodiethyl sulfide with the chlorides of palladium, gold, and other heavy metals—especially with palladium chloride—has been used for the identification of this compound. Reactions with salts of copper are not very characteristic under ordinary circumstances, but they can be modified to yield sensitive, rapid, and simple methods for the identification of 2,2′-dichlorodiethyl sulfide.

A sheet of filter paper saturated with a 2 per cent solution of copper sulfate in water, or a saturated alcoholic solution of copper nitrate, containing 1 to 2 per cent of glycol or glycerol, is laid horizontally on a few layers of clean filter paper, and dried at room temperature. The reactions are more distinct if the copper nitrate solution is used. Finally, the test paper is cut to size and stored in an airtight container.

The test paper is not affected by pure glycol. Commercial glycol containing triethanolamine phosphate slowly produces a blue ring. Thiodiglycol, the parent compound of 2,2′-dichlorodiethyl sulfide, produces almost immediately a bright green spot with a very marked green border; 0.1 to 0.4 mg. of thiodiglycol can easily be detected in this way. The sensitivity of the reaction is greater if the thiodiglycol is applied free of solvent.

Hydrochloric acid produces a very pale yellow spot, easily distinguished from the dark brown spot caused by a mixture of hydrochloric acid and 2,2′-dichlorodiethyl sulfide; the center of the brown spot gradually changes to violet. Less than 0.2 mg. of 2,2′-dichlorodiethyl sulfide can be recognized by this reaction.

The direct reaction of 2,2′-dichlorodiethyl sulfide is not entirely satisfactory at lower concentrations, but the following indirect reaction is satisfactory in every respect.

The sample suspected of containing 2,2′-dichlorodiethyl sulfide is extracted with ether, and the ether solution is refluxed with normal alkali on a water bath kept at about 60° C. for 30 minutes. If the amount of 2,2′-dichlorodiethyl sulfide is known to be very small, a few minutes' refluxing will suffice. A green color will appear on the test paper if more than 1 to 2 mg. of 2,2′-dichlorodiethyl sulfide is present.

To detect smaller quantities, the alkaline solution is acidified with dilute sulfuric acid in the presence of phenolphthalein, and the slightly acid solution is diluted with 5 volumes of alcohol. The precipitated sulfate is filtered and washed with absolute alcohol, and a few corns of anhydrous sodium carbonate are added to the filtrate to remove traces of acid. The test is positive if more than 0.1 to 0.2 mg. of 2,2′-dichlorodiethyl sulfide was originally present.

The test can be made still more sensitive by removing some or all of the solvent, but it is less sensitive than the sodium iodoplatinate test. Its practical advantage lies in the fact that the necessary reagents are easily procured and the test can be carried out in poorly equipped or emergency laboratories, or by A. R. P. workers who are not professional chemists.

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Determination of the Precision of Analytical Control Methods

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For the intelligent control of plant operations and product quality, it is essential that the precision of the analytical control methods be quantitatively known. The use of statistical reasoning based on the standard deviation has been found applicable. The analytical method is first tested under ideal conditions to find the highest precision of which the method is capable. If this precision is judged high enough, the method is then tested in routine practice for a year to discover the variability under routine laboratory conditions. An LU_2 (limit of uncertainty) can then be calculated within which routine analyses may be guaranteed. The average of duplicate determinations made at the same time does not result in as much improvement as may be theoretically calculated, evidently because the results are not truly random. Normal control methods were found to have 1.5 to 2.5 as much variation under routine conditions as the same method under the best conditions. The use of this method of criticism has proved a valuable tool in the author's laboratories.

IT IS a primary concept of nature that no one physical measurement is exact. Only those values that are accepted by definition are free from deviations in the last significant figure. The determination of the composition of any sample even by the best known technique is similarly influenced by the inability to measure weights, volumes, colors, chemical equilibria, etc., with exactitude. Practically all analytical methods contain enough small constant errors to make them somewhat empirical. It follows that some differences will be obtained between individual analytical results from the same sample even if the best possible technique and instrumentation are used.

When the conditions that obtain in commercial control laboratories are considered, the above concept becomes much more important, since the variations between personal analytical techniques, solutions, apparatus, and surrounding conditions are certain to influence the precision of an analytical method to a considerable extent. This is true even if the method is followed exactly as written; a departure from standard instructions or error in judgment would give further deviations from the truth.

Since these variations are known to exist, they are a constant threat to good commercial operation or product quality until they are quantitatively determined. An attempt to control a plant operation within 0.05 per cent of a standard value with a method precise to but ±0.5 per cent would obviously fail, yet similar situations frequently occur in industry because the precision of the analytical method is not known, There also exists a tendency on the part of many engineers. sales personnel, and even chemists to treat a single analytical result as an exact quantity and to make decisions therefrom that would be unjustifiable if the significance of the result were known.

After an extensive program of development and refinement of analytical methods, this company was faced with the determination of how well the routine control laboratories were following these new methods. Several years were spent in check sample work in which standard samples were run in duplicate by one laboratory and attempts were made to check these results in another laboratory. Although considerable information was obtained, a great many disputable differences arose that could not be traced to any assignable cause. One reason for this disagreement was found to be the use of the "average deviation" as a criterion, since any variations greater than the criterion were treated as poor analyses.

In commercial analytical practice speed is often essential for good control, even if accuracy and precision are sacrificed. It is often expedient to employ nontechnical personnel as analysts or testers for routine determinations. Any method for determining the precision of routine results must be able to evaluate these personnel factors.

The procedure described in this article was developed in the summer of 1940 and has been used throughout the company with satisfaction since that time.

TABLE I. PRECISION OF METHOD UNDER BEST CONDITIONS

Method: SC-11-3 of 6-27-41 for specific gravity of carbon tetrachloride by pycnometer)

Test No.	Specific Gravity at 25°/4° C.	d	d^2 ($\times 10^{10}$)
1	1.5849	−0.00016	256
2	1.5851	+0.00004	16
3	1.5853	+0.00024	576
4	1.5853	+0.00024	576
5	1.5850	−0.00006	36
6	1.5849	−0.00016	256
7	1.5847	−0.00036	1296
8	1.5851	+0.00004	16
9	1.5852	+0.00014	196
10	1.5851	+0.00004	16

Av. $= \overline{X}_1 = 1.58506$ $\Sigma d^2 = 3240 \times 10^{10}$

$$\sqrt{\frac{\Sigma d^2}{10}} = 0.00018 = \sigma_{10}$$

$$LU_{av.} = 1.58506 \pm \frac{3(0.00018)}{\sqrt{10}} = 1.58489 \text{ to } 1.58523$$

$$LU_1 = \frac{3(0.00018)}{0.923} = \pm 0.00059$$

Prior Concepts

A number of investigators (*2, 3, 5, 6*) have indicated that the variations of an analytical method may be treated by statistical calculations. Power (*6*) found that over 100 determinations had to be made before the variability of a micromethod for carbon could be accurately estimated. Informative as such an extensive investigation might be, such a study in a commercial laboratory would be difficult to justify for economic reasons. The two routines given below use 10 samples and 24 samples to obtain the precision and the results have been satisfactory from the author's point of view. Although some of the calculations have the surface appearance of complexity, the most complicated operation is the extraction of square roots.

The criterion by which the precision of the method is to be expressed is of considerable importance. It may be mathematically proved that the standard deviation, σ, is the most accurate measure of dispersion about an arithmetical mean. Any multiple of σ may be selected according to the limits of precision desired.

Since the author wished to express the precision as the limits within which an analytical result could be practically guaranteed, the value $\pm 3\sigma$ was chosen. If the variations are distributed about the mean according to chance and the determinations are representative, the average $\pm 3\sigma$ should include 997 out of 1000 determinations (*1, 7*). This is equivalent to one result outside the limit in a year's routine of daily analyses and was thought to represent the precision in the best possible manner for commercial practice. The investigator must use some judgment before any unreserved guarantees are given, since these characteristics of σ are strictly true only for a system of chance causes; but the viewpoint adopted was that all variations are due to chance until the cause becomes known. By intelligent usage of the data obtained from these calculations, variations due to other than chance causes may be detected and subsequently eliminated.

Although the accuracy of a method is of great importance, a general procedure for its determination cannot be described. When a standard sample made from known amounts of the desired ingredients can be prepared, a definite measure of accuracy may be determined, but such samples are sometimes impossible to create with certainty because of the nature of the sample. Most commercial methods are more or less empirical in nature and are accurate by definition, their purpose being to enable two or more interested parties to obtain substantially the same results on the same material when analyzed independently. All the author's methods were checked by whatever convenient means were possible, such as the use of standard samples, synthetic knowns, checks with standard methods, etc., and if the limit of uncertainty of the average (described below) bracketed the known result, the method was said to be "accurate" in a qualitative sense.

Limit of Uncertainty under the Best Conditions (LU_1)

The highest precision that can be expected from an analytical method is the precision as shown by the best available technician working under the most closely controlled conditions. The following procedure was prescribed as standard for obtaining a measure of this precision:

Prepare a synthetic mixture that exactly reproduces the commercial material and contains a known amount of the desired ingredient, or select a representative homogeneous sample of the commercial material. Store enough material for at least fifty analyses in containers that will prevent any change in composition for at least one year.

Select an analyst who is well acquainted with the method, preferably the one who developed the method.

Prepare fresh reagents, standardize all solutions, and calibrate all apparatus before making the determinations.

Run ten analyses as closely together as possible under the most favorable conditions. Calculate all results to one more place than is generally reported.

Calculate the arithmetical average, $\overline{X}_1$, of the ten analyses.

Calculate the standard deviation of the results by the use of the following equation:

$$\sigma_{10} = \sqrt{\frac{\Sigma d^2}{10}}$$

where σ_{10} = standard deviation from the average shown by the 10 results
d = individual deviation of each result from the average

Calculate the limit of uncertainty of the average, $LU_{av.}$, by the following equation (*1*):

$$LU_{av.} = \overline{X}_1 \pm \frac{3\sigma_{10}}{\sqrt{10}}$$

Calculate the limit of uncertainty of the method under the best conditions, LU_1, as follows (*1*):

$$LU_1 = \pm\frac{3\sigma_{10}}{0.923}$$

Table I indicates the method used for calculation.

If the sample is of known composition and the method is accurate, $\overline{X}_1 \pm LU_{av.}$ should bracket the known value. If the result differs, judgment has to be used to decide whether the method should be improved until it is accurate or a standard correction should be applied to all results. If the concentration is not known, the accuracy cannot be determined unless the method is "accurate by definition" as previously mentioned.

Since the LU_1 is a measure of the ultimate precision of the method as written, a decision as to the commercial applicability of the method can be made. If the indicated precision is high enough, the method is ready for test under routine conditions, but if it is found too low the method must first be improved, since the test under routine conditions is almost certain to show an even lower precision because of the additional sources of variation introduced.

Limit of Uncertainty under Routine Conditions (LU_2)

If all the above conditions have been satisfied, the method may be tested under routine conditions by the following prescribed procedure:

Use the sample on which the LU_1 test was made.

Have the sample analyzed in a routine manner by a routine technician, preferably together with routine plant samples of a

similar nature. If possible, the technician should not know the prior results.

Have the sample analyzed in duplicate each month for one year, using as many different routine technicians as possible.

As soon as the monthly analyses have been completed, compare the results with the average and LU_1 obtained during the first test. If the individual results are within $\bar{X}_1 + LU_1$, allow them to stand as representative. If the results are outside these limits, immediately check the technique, reagents, etc., so that any deviation from the method will be discovered before the details slip the technician's mind. If an objective difference in technique is found, have the analyses rerun by the same technician but in the correct manner. If no assignable cause is found, allow the results to remain uncorrected.

When the 12-month accumulation of 24 analyses has been completed, average the 24 results to obtain $\bar{X}_2$. Also calculate the average of each month's duplicate tests.

Calculate the standard deviation of the 24 individual tests, σ_{24}, and the standard deviations of the 12 monthly averages, σ_{12}.

Calculate the limit of uncertainty of the average as follows:

$$LU_{av.} = \frac{3\sigma_{24}}{\sqrt{24}}$$

Calculate the limit of uncertainty of the method (*1*):

$$LU_2 = \pm 3\,\frac{\sigma_{24}}{0.968}$$

Table II illustrates these calculations.

If the limit of uncertainty of the average includes the known value, the method may be called accurate. If the concentration is not known but the method is "accurate by definition", the limits of uncertainty of the averages under both LU_1 and LU_2 conditions must overlap for approval of the method. The LU_2 result is the objective figure of the whole study and represents the precision that may be expected from the method under routine conditions. Since 997 out of 1000 determinations can be expected to represent the truth within $\pm LU_2$, this result may be used to judge whether the method is precise enough for routine control and is a measure of the range within which analyses are to be trusted. If the LU_2 is judged small enough, the method is approved for routine but if it is too large for good control, it must be replaced or amended.

By comparison of the LU_2 with the LU_1, a measure of the amount of possible improvement in routine work is obtained. Since the LU_1 was obtained under ideal conditions, the LU_2 may be brought closer to the LU_1 value by further education of routine personnel, intensive standardization of technique or equipment, closer temperature control during seasonal variations, etc., but it might be found that the analytical method possesses inherent characteristics that allow personal variation that cannot be standardized without extensive modification of the method. The comparison of the standard deviations shown by the individual analyses and the duplicate averages represent the amount of improvement that may be obtained by the averaging of two or more analyses. For all these possibilities that may confront the investigator, the LU_1 and LU_2 are excellent tools for guidance.

Table II. Precision of Method under Routine Conditions

(Method: SC-11-3 of 6-27-41 for specific gravity of carbon tetrachloride by pycnometer. Average of 10 analyses for LU_1 test = 1.58506, LU_1 = ±0.00059)

Month	Analyst	Specific Gravity at 25°/4° C.	Average of Duplicates	Deviation of Individual from Grand Average, d_1	Deviation of Duplicate Average from Grand Average, d_2
January	1	1.5850		−0.00003	
	1	1.5851	1.58505	+0.00007	+0.00002
February	2	1.5848		−0.00021	
	2	1.5850	1.58490	−0.00003	−0.00013
March	1	1.5846		−0.00043	
	1	1.5846	1.58460	−0.00043	−0.00043
April	2	1.5851		+0.00007	
	2	1.5851	1.58510	+0.00007	+0.00007
May	3	1.5853		+0.00027	
	3	1.5853	1.58530	+0.00027	+0.00027
June	4	1.5849		−0.00013	
	4	1.5850	1.58495	−0.00003	−0.00008
July	5	1.5849		−0.00013	
	5	1.5848	1.58485	−0.00023	−0.00018
August	3	1.5854		+0.00037	
	3	1.5854	1.58540	+0.00037	+0.00037
September	3	1.5852		+0.00017	
	3	1.5853	1.58525	+0.00027	+0.00022
October	2	1.5847		−0.00033	
	2	1.5851	1.58490	+0.00007	−0.00013
November	2	1.5850		−0.00003	
	2	1.5850	1.58500	−0.00003	−0.00003
December	6	1.5849		−0.00013	
	6	1.5851	1.58500	+0.00007	−0.00003

Grand average = $\bar{X}_2$ = 1.58503

Standard deviation (individual determinations) = $\sigma_{24} = \sqrt{\frac{\Sigma d_1^2}{24}} = 0.00022$

Standard deviation (duplicate averages) = $\sigma_{12} = \sqrt{\frac{\Sigma d_2^2}{12}} = 0.00021$

Limit of uncertainty of grand average = $1.58503 \pm \frac{3\sigma_{24}}{\sqrt{24}}$ = 1.5849 to 1.5852

Limit of uncertainty of method under routine conditions = $\pm\frac{3\sigma_{24}}{0.968}$ = ±0.00068 = LU_2

Ratio of improvement by duplication = $\frac{\sigma_{24}}{\sigma_{12}} = \frac{0.00022}{0.00021} = 1.05$

Ratio of $\frac{LU_2}{LU_1} = \frac{0.00068}{0.00059} = 1.15$

Discussion of Results

The analytical method reported in Tables I and II represents a simple technique that is of major importance to the author's laboratories, since it is a sensitive test for the composition and purity of chlorinated hydrocarbons and their mixtures. This determination is made in a 25-ml. pycnometer containing an accurate thermometer. The pycnometer is filled with the sample and allowed to expand to near equilibrium temperature in a balance room. The contents are adjusted to the mark, the temperature is read, and the pycnometer is weighed. The indicated specific gravity is then corrected to 25°/4° C. by means of the appropriate factor.

The LU_1 test (Table I) indicated that this method would give results within ±0.0006 of the mean in 997 out of 1000 trials. By comparison with plant control and finished product specifications, this indicated precision was found satisfactory. The average and limit of uncertainty of the average check closely with the 1.5850 at 25°/4° C. characteristic calculated from reported data for carbon tetrachloride (*4*). Accordingly, the method was judged ready for the LU_2 test.

The LU_2 test (Table II) indicated that the method was also accurate in routine work, since the average agreed very closely with the previously obtained figure. The LU_2 was found to be ±0.0007, which was only slightly higher than the LU_1 of ±0.0006 and represents very good agreement for routine work. The method was therefore judged satisfactory for all routine laboratory work.

The standard deviation of the averages of the duplicate determinations in this study was most enlightening. If the deviations were purely random, the averaging of two independent analyses should have decreased the variations by a factor of $\sqrt{2}$ or 1.41. The actual improvement as shown by the ratio of standard deviations was only 1.05. This ratio indicated that duplicate analyses made at the same time are not truly random but are rather influenced by slight variations in technique or surrounding conditions.

Table III gives the over-all results from a number of representative commercial analytical methods. For each of these seven methods the precision shown by the LU_1 test

appeared satisfactory. The LU_2 of tests 1, 3, 4, and 5 were low enough for the required closeness of control. Test 2, a determination by a modified Volhard method for chlorides, indicated an LU_2 that was 2.7 times as large as the LU_1 and was considered too variable for good control. This method was re-examined for possible improvement. Tests 6 and 7 were obtained by a refined Engler distillation technique, but the results were found to be influenced by seasonal variations and superheating of vapors in the flask. These methods were also improved to eliminate these assignable causes.

Commercial Application

Because the study is founded on statistical reasoning from the comparatively small number of samples dictated by economic reasoning, the results are open to some variations in an absolute sense. However, in practice this objection has been found academic, since the study has resulted in definite precision characteristics close enough for purposes of refinement of methods and techniques and to discover within what limits routine analytical results are significant. The procedure described is offered as a tool for such investigation and as a standard method for describing precision.

Table III. Precision Determinations of Representative Commercial Analytical Methods

No.	Sample	Analysis	LU_1	LU_2	LU_2/LU_1	Improvement Ratio of Duplicate Determinations
1	50% caustic soda	NaOH, %	±0.078	±0.147	1.9	1.2
2	50% caustic soda	NaCl, %	±0.0102	±0.0280	2.7	1.0
3	50% caustic soda	Fe, p. p. m.	±0.77	±1.00	1.3	1.1
4	Carbon tetrachloride	Sp. gr. at 25°/4° C.	±0.00059	±0.00068	1.15	1.05
5	Carbon bisulfide	Nonvolatile residue, %	±0.0004	±0.0008	2.0	1.0
6	Trichloroethylene	Boiling range, ° C.	±0 11	±0.33	3.0	1.07
7	Trichloroethylene	First drop, ° C.	±0.05	±0.30	6.0	1.07

In the study of some 40 basic analytical methods during the last two years, the following relationships were found from the LU_2/LU_1 ratio:

Ratios of 1.0 to 1.5 indicate that the variations result mainly from limitations of instrumentation.

Ratios of 1.5 to 2.5 indicate normal relationship between analyses made under the best conditions by one man and analyses made under routine laboratory conditions.

Ratios over 2.5 generally indicate considerable personal and seasonal variations that may be reduced without modification of the basic analytical method.

Duplicate determinations run at the same time are generally not truly random and the averaging of such results does not improve the precision appreciably. The only justification for running analyses in duplicate is the additional safeguard afforded against an outright mistake, such as an error in calculation or an erroneous weighing.

Most commercial analyses are made on products of approximately constant composition, so that a measure of precision may be expressed in terms of the actual percentage variations on the original sample basis. Whenever several widely different levels of composition exist, it has been found desirable to make complete studies at each level to discover whether or not the extent of the variations is proportional to the amount of ingredient.

Procedure for Decomposable Samples

Several cases have been encountered where the year's study was rendered impossible because of the instability of the samples or attack on the containers. In this case either one of two techniques has been used to approximate the true LU_2:

1. If the sample decomposes at a constant rate, the results may be plotted against time and an average curve drawn through the points. The deviations of the individual results from this curve are used for the calculation.
2. If the sample decomposes in an inconstant manner or attacks its container appreciably, or if an approximate determination of the routine precision is desired rapidly, a speedup of the technique may be used by making the LU_2 tests during one day by as many routine analysts as possible. This gives only an approximation of the true LU_2, because variations encountered during a yearly study may not be observed. It does yield an approximation of personal differences.

The use of standard samples in the control laboratory has the additional advantages of providing periodical checks on personal techniques and for the education of new laboratory personnel. Since the basis of comparison is fair, the individual technician is more likely to cooperate than if he is expected to duplicate exactly a single analysis from some outside source.

These techniques have been in use throughout this company for over two years and have formed the first common bases for describing and comparing precision. They have led to the discovery and subsequent correction of many variations previously not suspected and the quantitative evaluation of other deviations that were known to exist. By the judicious use of LU_2 figures, the men having supervision of plant operation and shipping know within what limits routine analyses are to be trusted. Some similar procedure could well be adopted as standard by industrial analytical chemists to provide a mutual basis of understanding when describing the precision of analytical methods.

Acknowledgment

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Turbidimetric Determination of Small Amounts of Chlorides

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A turbidimetric method, based on the Tyndall effect, is given for the determination of small amounts of chloride. The method is essentially one in which the nephelometric method has been adapted to the Hellige turbidimeter in order to eliminate the disadvantages of reference standards. The method is rapid, requiring less than 15 minutes' working time per determination, and is readily adaptable to routine work. The accuracy is comparable with that obtained with the nephelometer.

THE first accurate measurements on silver chloride suspensions were made nephelometrically by Richards and Wells (*3*). Lamb, Carleton, and Meldrum (*2*) later modified the earlier procedure for the preparation of the suspensions, and the method which they adapted to routine analysis has been put into general use (*5*). Kolthoff and Yutzy (*1*) made a complete investigation of this method and a systematic study of the various factors affecting the results of nephelometric chloride determinations. From the results obtained by these and other workers, a tried and tested nephelometric method has been evolved.

The nephelometer was designed originally to surmount the difficulties incurred in gravimetric and volumetric methods for small quantities of precipitate. When applied to small amounts of chloride, the nephelometric method is admittedly accurate; but the preparation of fresh standards for each determination has been found inconvenient and time-consuming. Certain permanent, artificial standards such as kieselguhr, ground or etched glass, etc., have been used, but only with limited success. Since the advent of the nephelometer, micro and semimicro techniques have been developed; and principally because of the difficulties involved in the preparation of standards, low chlorides have most often been determined by either microgravimetric or microvolumetric methods. Both methods are time-consuming and require considerable equipment and special technique; as a result they are not readily adaptable to rapid routine work.

It is the purpose of this paper to show how the nephelometric method may be used with the Hellige turbidimeter to give a rapid and reliable method for the determination of small amounts of chloride. The method is accurate, and simple enough to be used by one unskilled in the use of elaborate and expensive equipment. It is rapid, requiring less than 15 minutes' working time per determination. A standard curve (Figure 1), covering the full range of the instrument, may be prepared in a few hours, and eliminates the need of reference standards. The instrument is easy to operate, and unique inasmuch as it combines the principles of the turbidimeter and the nephelometer. Its range is greater than that of the turbidimeter and equal to that of the nephelometer, and its use does not necessitate the preparation of fresh standards for each determination. This greatly simplifies the procedure by eliminating possible errors due to faulty preparation of individual standards, and also reduces the working time to less than one-half that required for the nephelometer.

The turbidimeter used in this investigation compares a beam of light with the Tyndall effect produced from a lateral illumination of the specimen by the same light source. The beam of light appears to the observer as a circular spot in the center of the Tyndall effect of the illuminated liquid, which is seen as lighter or darker than the Tyndall light, depending on the size of the opening of a precision slit. By matching the brightness of the two fields, the apparatus may be calibrated over a complete range of chloride concentrations. The construction and operation of this instrument for measuring the turbidity of barium sulfate suspensions have been given in complete detail by Sheen, Kahler, and Ross (*4*).

Reagents

Stock chloride solution, 4.12 grams of C. P. sodium chloride per liter, in distilled water.

Diluted chloride solution, 10 ml. of the stock solution diluted to 1 liter with distilled water. This solution contains 25 mg. of

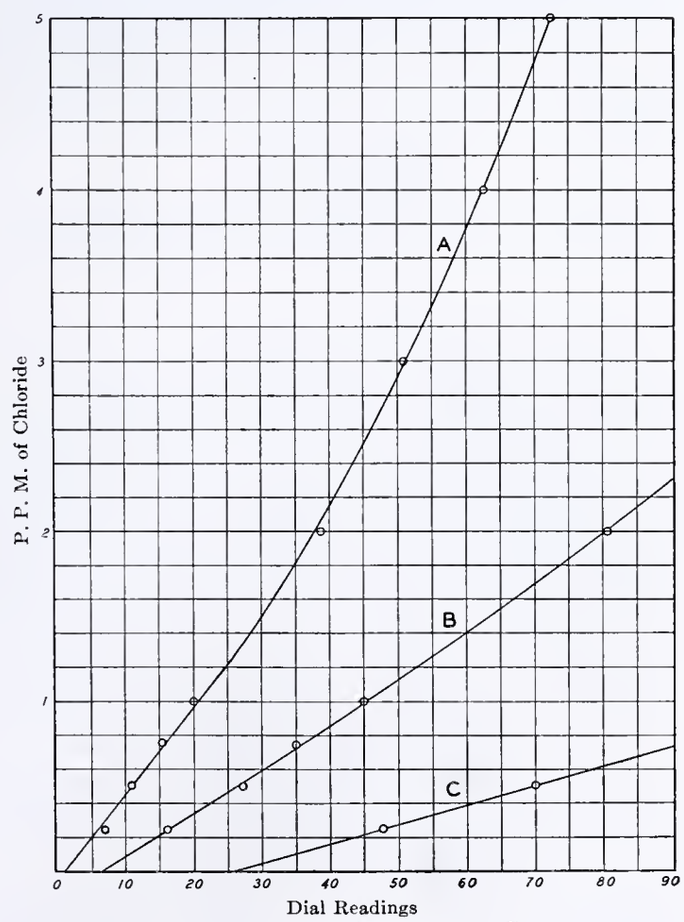

FIGURE 1. TYPICAL CALIBRATION CURVES

chloride per liter; 1 ml. diluted to 50 ml., as in the procedure, gives a solution corresponding to 0.5 p. p. m. of chloride.

Nitric acid–silver nitrate reagent. A solution approximately 0.2 *N* in nitric acid and 0.01 *N* in silver nitrate is prepared by diluting 12.5 ml. of concentrated nitric acid to 1 liter and adding 1.70 grams of silver nitrate.

Absolute ethanol. (Standard curves have also been prepared using 95 per cent and Formula 30 alcohols.)

A blank determination must be made in order to eliminate the possibility of chloride contamination from the reagents.

Table I. Standardization of Instrument

(20-mm. optical cell)

Chloride	Standard Solution	Dial Readings		
		No filter	Milk filter	Gray filter
P. p. m.	*Ml.*			
5	10	72–73	...	..
4	8	62–63	...	..
3	6	51–52	...	..
2	4	36–37	80–82	..
1	2	20–21	45–46	..
0.75	1.5	15–16	35–36	..
0.50	1.0	12	26	70
0.25	0.5	7	16	48
Blank on reagents	...	...	8	21

Procedure

Transfer the sample to a 50-ml. volumetric flask, adjust the pH with either 1 *N* nitric acid or 1 *N* sodium hydroxide until the solution is just neutral to phenolphthalein, and add distilled water until the volume approximates 20 ± 1 ml. Add 20 ml. of absolute ethanol and then, dropwise from a pipet, 5 ml. of the nitric acid–silver nitrate reagent while swirling the contents of the flask. Make up to the mark with absolute ethanol and shake. If any initial turbidity is found before the addition of the nitric acid–silver nitrate reagent, the sample must be rejected, because turbidity at this point indicates contamination.

Place the volumetric flask in a water bath at 40° C. for 30 minutes, and then cool the sample rapidly to room temperature. Pour into a 20-mm. optical cell (a manufacturer's accessory for the turbidimeter), stir in an additional 10 ml. of absolute ethanol, and determine the turbidity as soon as the bubbles cease to form in the cell. The sample should be read within 30 minutes after it has been cooled to room temperature. The reading is compared with the standard curve, and the amount of chloride calculated.

Calculation

$$\text{Per cent of Cl} = \frac{\text{p. p. m. of Cl (from the curve)} \times 0.005}{\text{weight of sample, in grams}}$$

Discussion

The standard curves were prepared from values obtained when known volumes of the standard chloride solution were treated in the same way as the sample. The dial readings were taken and these values plotted. Table I gives the results obtained, and Figure 1 shows a set of curves obtained in a typical calibration. Curve A represents the complete range of the instrument, 0 to 250 micrograms of chloride, and was obtained without the use of a filter. Suspensions of silver chloride made by the above procedure, which contain more than 250 micrograms of chloride, are too opaque to be read accurately with the instrument. Sample sizes taken must be such that the upper limit of the chloride concentration is not exceeded. Curve B was obtained by using the milk-glass filter, and curve C with the gray glass filter (both filters supplied as manufacturer's accessories). From the curves it follows that suspensions of less than 2 p. p. m. can be read more accurately on curve B than on curve A. Curve C may be used for concentrations of less than 1 p. p. m.

The accuracy to which dial readings may be reproduced determines, in part, the accuracy of the method. It has been found that dial settings may easily be made to within ±1 unit, by either the same or different operators. This limits the maximum deviation to less than 3 per cent for all concentrations of chloride, which is comparable with the accuracy obtained with the nephelometer.

Kolthoff and Yutzy (*1*) determined the effect of certain foreign ions on the magnitude of opalescence of silver chloride suspensions. They found that different ions have different effects, and these changes should be taken into account when preparing standard curves. If the instrument is to be used for routine work, standard curves should be prepared by taking a given volume of the diluted standard chloride solution, adding an amount of purified electrolyte equivalent to that in the sample to be investigated, diluting to 20 ml., and then following the procedure given above. In this way accurate curves may be prepared for each compound in which the chloride content is being determined. In many instances, however, chloride concentrations of 0.005 per cent or less are of interest to an accuracy of only one significant figure, and a special reference curve is unnecessary.

The volumes of the reagents used must be the same as those given in the procedure, because the alcohol serves to decrease the intensity of the opalescence as well as to stabilize the suspension. The final 10 ml. of alcohol which are added have no effect on the suspension other than dilution, but are added to adjust the solution to the proper depth in the 20-mm. optical cell. Other factors which affect the opalescence are heating, time of standing, mixing, and relative concentrations. Lamb and co-workers (*2*) found that the intensity approached a maximum upon heating the suspension for 30 minutes at 40° C., and at that point it remained stable for an hour. Higher concentrations are stable for about 0.5 hour. The suspension should be mixed just before it is placed in the water bath and just before it is read, as continuous mixing will cause the silver chloride to coagulate.

At present this instrument is being used in conjunction with the lamp combustion method for the routine analysis of organic compounds for small amounts of chloride and sulfur. It may also be used to determine the chloride content of wash liquors, and for the routine checking of many inorganic compounds which contain traces of chloride.

The limited solubility of certain compounds in 1 to 1 water-alcohol mixtures makes the use of acetic acid solutions necessary. Good results may be obtained when acetic acid is substituted for alcohol, and the standard curves may be prepared by the same procedure as given above.

Table II. Limits of Accuracy of Instrument

Chloride	Filters		
	None	Milk	Gray
P. p. m.	*P. p. m.*	*P. p. m.*	*P. p. m.*
5	±0.11	....	....
4	±0.10	....	....
3	±0.08	....	....
2	±0.06	±0.04	....
1	....	±0.04	....
0.75	....	±0.04	±0.01
0.50	....	....	±0.01
0.25	....	....	±0.01

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Color Index

Light-Colored Petroleum Products

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The applicability of the system tentatively known as "photoelectric color" to the evaluation of light-colored petroleum products has been demonstrated, thus making possible a continuous color scale for all petroleum products.

This color index consists of two parameters which are read directly on a special photoelectric instrument. The two parameters completely designate the color of materials such as oils in terms which are of direct technological significance and which express directly the appearance values.

Light-colored oils are read with a violet filter instead of North Sky and red and converted to this color index by a suitable equation, so that no change in sample depth is required. For light oils, the second parameter is zero.

I. C. I. data may be calculated from the color index if desired, although this is usually unnecessary in petroleum technology.

The Saybolt chromometer has been shown to be unreliable, and to be importantly affected by the surface tension, refractive index, and specific dispersion of the oil being tested.

This color index may be converted to Saybolt color by the establishment of suitable curves for the types of product in question. The conversion is prepared statistically to allow for the unreliability of the Saybolt method.

The required colorimeter can be adjusted during manufacture to the specified standard, so that the readings obtained are independent of one's particular instrument.

THE color of petroleum products is of importance in their processing and marketing. Hitherto, for its measurement, the petroleum industry has employed several systems, all of which are visual, and depend upon matching the color of the sample with that of a standard glass. Committee D-2 of the A. S. T. M. on Petroleum Products and Lubricants specifies two such methods, the Union colorimeter (*4*) for lubricating oils and the Saybolt chromometer (*5*) for light-colored products. The color of oils customarily measured with the Union colorimeter has been the subject of a previous paper (*11*), which described a system for color designation and measurement. It is the purpose of this paper to discuss in a similar manner the colors of oils usually determined with the Saybolt chromometer, and to propose means for handling them by the method already described (*11*).

The Saybolt chromometer consists essentially of two glass tubes 50 cm. (20 inches) in length, which are illuminated from the bottom by light reflected from a mirror. A prismatic optical head provides a circular field of vision, one half of which is illuminated by the light from the sample, and the other by light passing through the color standard. The level of the oil in the sample tube is adjusted so that its color matches that of the standard disk in the other tube. The height of oil is read and is converted to Saybolt chromometer color by an arbitrary table. The instrument, invented by George Saybolt, has been brought to its present stage of standardization largely as the result of A. S. T. M. activity (*1, 2, 3, 7*).

Both the Union colorimeter and the Saybolt chromometer are arbitrarily standardized and are not related to one another by any fundamental means. Furthermore, the specifications of each of these instruments are so drawn that sufficient latitude is allowed in the selection of color disks to permit an appreciable variation of results from instrument to instrument. For these reasons, among many others, the industry has needed one system of color measurement which will be applicable to all types of petroleum products, and which will provide a continuous relationship between the colors of dark- and light-colored products. Several attempts of this nature have been made prior to and since the development of the Saybolt instrument, leading to the development of the Stammer colorimeter (*17, 25*), methods of using the Lovibond glasses (*6, 16, 18, 20, 21*), and color systems known as "true color" (*23, 24, 26, 28*), and "optical density" (*12*).

All these methods failed in their objective for one reason or another. None of them is directly related to accepted systems of color definition (*14, 19, 22*), which, with several others, have been described by Gardner (*13*). All are not only subject to peculiarities of the individual instrument, but lean heavily upon the personal equation. Furthermore, for light-colored oils, these systems require cell depths appreciably greater than used for darker oils, which prevents use of a continuous color scale. The Stammer and the Duboscq type colorimeters (*15*), in which the depth of oil is varied until a standard is matched, suffer from the same faults, including the variations in hue and, in addition, frequent failure of Bouguer's law to apply to petroleum products. "True color" has certain advantages, but is frequently invalidated by comparison with dilutions of oils of different hue, or because Beer's and Bouguer's laws do not hold for such dilutions. (According to Bouguer's law the logarithm of the reciprocal transmittance is proportional to depth: $\log_{10}(^1/_T) = kd$.)

Story and Kalichevsky (*27*) proposed the use of a photoelectric colorimeter, primarily as a substitute for the eye. This eliminated the subjective nature of color measurement of petroleum products.

A new color index for the designation and determination of color, previously called "photoelectric color" has been proposed (*11*), and the designation of the colors of lubricating oils and darker colored petroleum products by means of this index has been described. "Photoelectric color" was originally chosen for want of a better name to distinguish this system of color designation and measurement from Lovibond,

Saybolt, or Union, but it is probable that the industry will find one which is more appropriate as this color index becomes accepted.

This color index conveys full color information concerning the oil. In addition to presenting this information in terms which are of immediate and direct significance in petroleum technology, advantage is taken of the substantially invariant color properties of these series of products—that is, dark-colored petroleum products, light-colored petroleum products, fatty oils (*9*), etc. By so doing, all the desired information can be given despite the use of less than three parameters, in the present instance, only one parameter.

The color index consists of two terms, the second being zero when the color is "normal" or when it is that of light-colored petroleum products. The first term is the North Sky reading (or its computed equivalent in the case of light products as shown below), and the second is the deviation of the red reading from the normal at that North Sky reading. This normal is obtained from Figure 3 of another paper of this series (*11*). By reporting deviation from the normal red reading rather than the red reading itself, one can tell at a glance whether the oil is greenish or reddish and to what extent. For example, a color index of 90 + 3 would represent a North Sky reading of 90 and a red reading of 100 and the oil is on the reddish side of normal. An index of 90 − 3 represents a North Sky reading of 90 and a red reading of 94 and the oil is greenish to the extent of 3 units. When the second term is zero, it will often be omitted, as in Figure 6, a chart for the conversion of this color index to Saybolt color.

This system expresses and measures directly what the eye sees. It does so by measuring the equivalent of the energy received by the average eye looking through the sample toward C illuminant, and it also measures variations in hue. While it serves for darker oils, modification was necessary in the case of light-colored products, to magnify the readings without changing (increasing) sample depth.

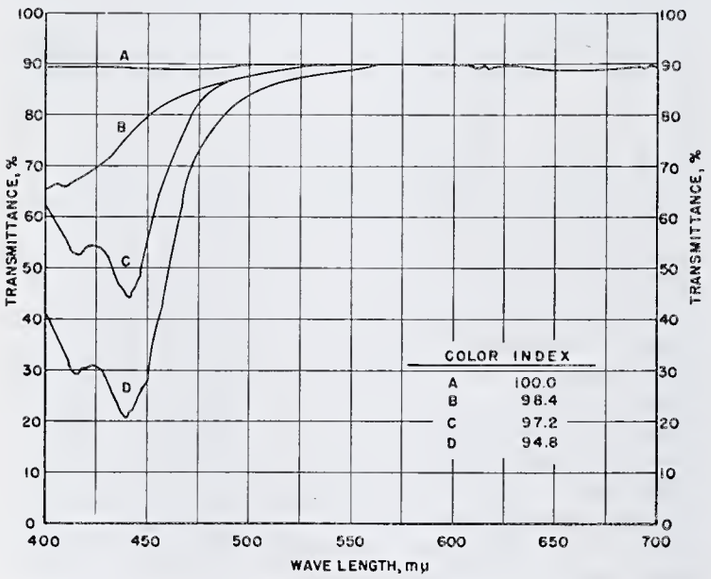

FIGURE 1. TYPICAL SPECTROPHOTOMETRIC CURVES FOR SOLVENTS

One way to magnify the reading would be to increase the sample depth appreciably above the present 1.8 cm. However, this increase would be objectionable because (1) errors due to bubbles and turbidity would be equally magnified; (2) the energy loss due to fluoroscence would be increased; (3) Bouguer's law would not hold rigidly, owing to the effect of fluorescence and other factors; and (4) it would entail redesign of a successfully simplified instrument with

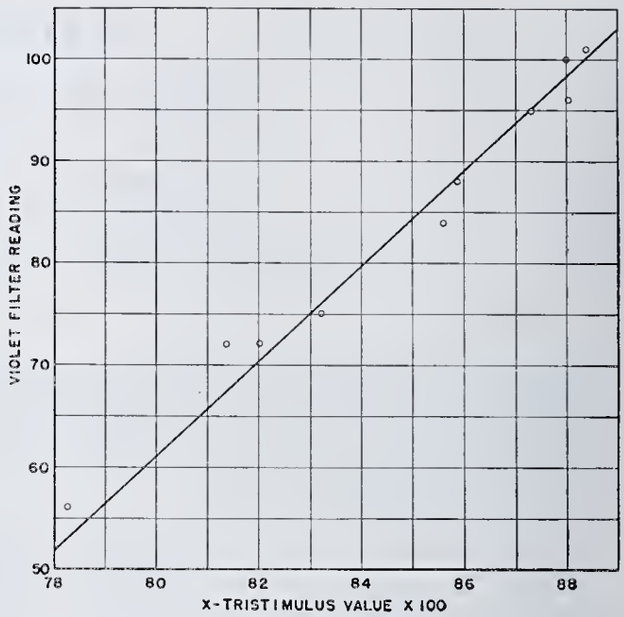

FIGURE 2. CONVERSION CHART, VIOLET READINGS TO X

consequent increases in complexity, cost, and likelihood of error. The use of two instruments, as is present practice, would be even more objectionable.

In the case of light-colored petroleum products, the eye "sees" and is influenced almost exclusively by light transmission of the oil in the violet range. Accordingly, magnification of the reading, without change in sample depth, can be accomplished by the use of the violet filter. Readings of light-colored petroleum oils with this filter have a direct relationship with those made with the North Sky filter. Thus, the disturbing effects of turbidity, bubbles, and fluorescence are not equally magnified, and no reliance need be placed upon the applicability of Bouguer's law. One instrument, therefore, provides a continuous color scale for all petroleum products. A further advantage lies in the fact that the color of these products can be obtained with less than 10 ml. of sample, instead of the 50 ml. or more required for other methods.

In the range of darker oils, no monochromatic filter could be found which was the equivalent of the North Sky filter. However, with lighter colored oils, the changes in the spectral curves are confined to the portion below 550 mμ with virtually no absorption above this wave length. Curves for these oils are smooth in shape and show greater transmittance as the wave length increases. The ultimate is a curve for a water-white oil which consists substantially of a straight line at 90 per cent transmission. As these water-white oils oxidize and darken in color, their spectral curves deviate from this line, but only in the region below 550 mμ. This is illustrated by Figure 1, showing the progressive darkening of a water-white oil. Thus, the average eye, in viewing C illuminant through these light-colored oils, is influenced only by changes in the blue and violet region, so that a measure of the absorption below 550 mμ is all that is required. The instrument with the violet filter in position has an excellent response in this region, and thereby performs the same function for light-colored oils that the North Sky filter does with darker ones. Indeed, the authors' experimental work has confirmed that the relationship between violet and North Sky filter readings in this range is linear.

A further peculiarity of light-colored petroleum products, as distinguished from darker ones, is that because of the high degree of refining given them there is a negligible light

absorption at wave lengths greater than 550 mμ. Also, for oils of the same color index, there is no appreciable difference in the steepness of their spectral curves. Hence, only one parameter suffices for a full description of the color and the red reading for determination of variations of hue is not required.

Definitions

The terminology used by the petroleum industry in dealing with light-colored products is not rigidly standardized. Therefore, for the purpose of this discussion, the following definitions have been established:

Refined Oil. Any oil which has been rendered light in color (straw color to colorless).

Kerosene. Any refined oil suitable for use as an illuminant in a wick lamp.

Solvent. Any refined oil of narrow boiling range suitable for solvent purposes.

Used Oil. Any oil suitable as a lubricant which has been subjected to service conditions.

Description of the Method

As has been described (*11*), the color index of dark oils is taken with the North Sky filter, and optionally with the red filter. For the reasons already given, refined oils must be measured with the violet filter, which gives readings related to those found with the North Sky filter, and thus provides a continuation of the scale. This filter was so chosen that, with paraffinic kerosene and paraffinic solvents, it gives exactly ten times the deflection obtained with the North Sky filter. Thus, the equivalent North Sky reading of these products is computed, using the equation:

$$\text{N. S.} = 90 + \frac{\text{violet filter reading}}{10}$$

For values up to 96.0 the North Sky filter is used, but above 96.0 the violet filter and the above equation are employed. Readings are taken to the nearest half-scale division, and the first term of the color index is reported to the nearest 0.05 unit. Thus, an oil with a violet filter reading of 78.5 will have a color index of 97.85.

Table I. Color Data on Refined Oils

Oil	Nature	Spectrophotometric X	Y	Z	Color Index N. S.	V
1	Paraffinic	0.880	0.899	1.056	100.0	100.0
2	Aromatic	0.884	0.903	1.051	102.0	101.0
3	Aromatic	0.880	0.903	1.026	100.5	96.0
4	Paraffinic	0.873	0.899	1.022	100.0	95.0
5	Aromatic	0.859	0.895	0.941	100.0	88.0
6	Paraffinic	0.856	0.892	0.938	99.0	84.0
7	Paraffinic	0.832	0.898	0.801	98.0	75.0
8	Paraffinic	0.820	0.890	0.752	97.5	72.0
9	Aromatic	0.814	0.884	0.709	98.0	72.0
10	Aromatic	0.783	0.879	0.527	96.0	56.0
11	Paraffinic	0.778	0.876	0.526	94.0	48.0

Since the colors of certain types of refined oils are highly unstable, and are markedly affected by exposure to light, it is essential that measurements be taken as quickly as accuracy permits. Because of the bleaching action of the light beam, the color of the sample is lightened appreciably in the vicinity of the focal point of this beam. The oil in the sample tube should, therefore, be agitated between check readings to disperse the bleached material. The instrument enables measurements to be made at the rate of six samples per minute.

Experimental

Colorimeter and Filters. For this work the colorimeter already described (*11*) and based on Diller's (*8, 10*) was employed. In order to extend the proposed system to light-colored oils, the same filters were used. However, since such oils have practically identical spectral curves above 550 mμ, and there are but slight differences in their hues, the response of the red filter was substantially the same in all cases; so that this filter reading is not required. The same reference standard used previously (*11*)—namely, distilled water—was employed throughout, and the colorimeter was set by this standard to a scale reading of 100 prior to all determinations.

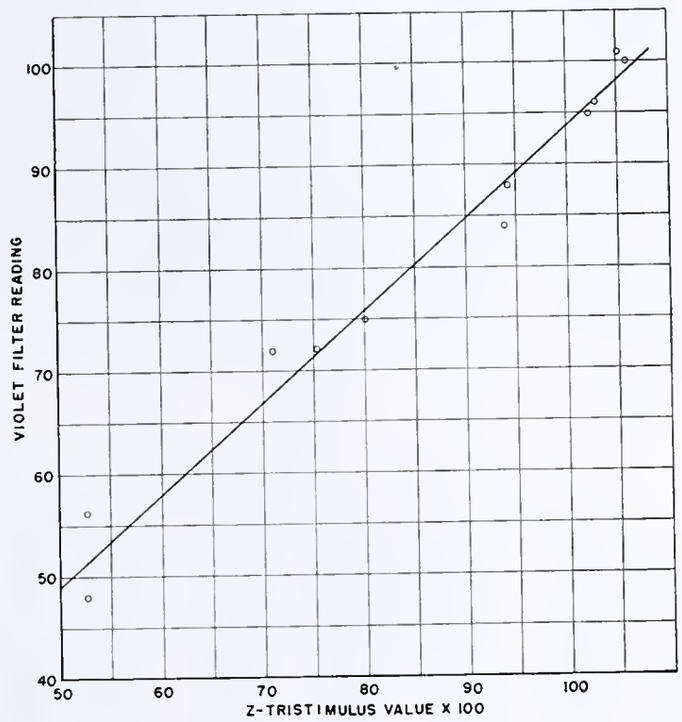

Figure 3. Conversion Chart, Violet Readings to Z

As has been previously emphasized, the instrument as a whole, and not merely the filters alone, must be designed and manufactured to yield the standard readings. The temperature of the light source, characteristics of the photocell, optical system, etc., as well as the filter determine the response. Of particular importance to this work is the fact that the instrument is so designed as to minimize the effects of variations in refractive index and fluorescence of the sample.

Correlation with I. C. I. Values. As in the previous investigation, the readings of the colorimeter can be correlated directly with I. C. I. data. This was accomplished by having eleven representative oils evaluated by the Electrical Testing Laboratories with a Hardy spectrophotometer, and obtaining data simultaneously with the photoelectric colorimeter. These samples represented two series of refined oils having widely differing physical characteristics, one being of paraffinic and the other of aromatic nature. The spectrophotometric data were converted to tristimulus values by the 30 selected ordinates method described by Hardy (*14*). These values and readings taken with the two filters are shown in Table I.

The conversion of North Sky filter readings to Y tristimulus values has already been established (*11*), and the conversion factor of 0.90 was used here also. This factor results from the use of distilled water, which has a transmission of 90 per cent as the standard for the color index.

In order to obtain conversions to X and Z, the values obtained spectrally were plotted against violet filter readings as shown in Figures 2 and 3. Figure 2 is the more sensitive curve mentioned in the previous paper (*11*) for obtaining

the X tristimulus value for light-colored oils. The most representative curves for these points were straight lines. It is logical to expect that such correlations exist, since, as already shown, the only variations in the spectrophotometric curves occur at wave lengths below about 550 mμ. In this range, readings with the violet filter, as well as the Z function and a portion of the X function, are affected. This relationship is further confirmed by Figure 4 wherein for these eleven representative samples X is plotted against Z, which results in a straight line. Using Figures 2 and 3, X and Z tristimulus values were obtained from the violet filter readings. These and the Y tristimulus values obtained as above are given in Table II. The mean deviation of the authors' values obtained spectrophotometrically is shown to be ±0.012.

The trichromatic coefficients were calculated from the tristimulus values, and are shown in Table III. A mean deviation of ±0.004 is indicated. Finally, from Y, x, and y the luminous transmission, dominant wave length, and purity were obtained. These data are shown in Table IV, wherein mean deviations from the true values of ±0.9 per cent in transmission, ±3 mμ in dominant wave length, and ±1.5 per cent in purity are found.

Table II. Tristimulus Values

	$X \times 100$		$Y \times 100$		$Z \times 100$	
Oil	Spectral[a]	Color index[b]	Spectral[a]	Color index[b]	Spectral[a]	Color index[b]
1	88.0	88.4	89.9	90.0	105.6	106.5
2	88.4	88.6	90.3	91.8	105.1	108.0
3	88.0	87.5	90.3	90.5	102.3	102.5
4	87.3	87.3	89.9	90.0	102.2	101.4
5	85.9	85.8	89.5	90.0	94.1	93.5
6	85.6	85.0	89.2	89.1	93.8	89.0
7	83.2	83.0	89.8	88.2	80.1	78.8
8	82.0	82.4	89.0	87.8	75.2	75.6
9	81.4	82.4	88.4	88.2	70.9	75.6
10	78.3	78.9	87.9	86.4	52.7	58.0
11	77.8	77.2	87.6	84.6	52.6	49.0

Mean deviation from spectra ±1.2

[a] Computed from Hardy spectrophotometric curves.
[b] Converted from readings of photoelectric colorimeter.

The above may be summarized as indicating that these color indices for refined oils may be converted to I. C. I. values with reasonable accuracy. This correlation is only for petroleum oils, and is possible because their spectral curves have the same generic shape.

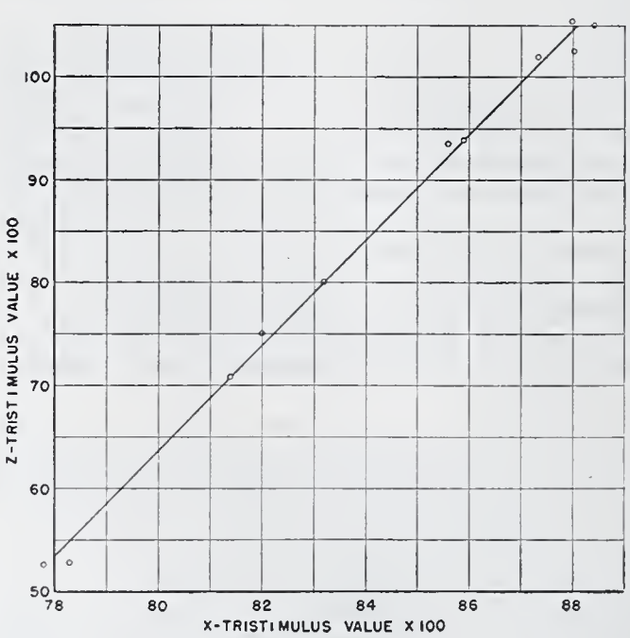

Figure 4. Relationship of X and Z

Table III. Trichromatic Coefficients

	x		y		z	
Oil	Spectral	Color index	Spectral	Color index	Spectral	Color index
1	0.310	0.310	0.317	0.316	0.373	0.374
2	0.311	0.307	0.318	0.318	0.371	0.375
3	0.313	0.312	0.322	0.323	0.365	0.365
4	0.312	0.313	0.322	0.323	0.366	0.364
5	0.319	0.319	0.332	0.334	0.349	0.347
6	0.319	0.323	0.332	0.339	0.349	0.338
7	0.329	0.332	0.355	0.353	0.316	0.315
8	0.333	0.335	0.362	0.357	0.305	0.308
9	0.338	0.335	0.367	0.358	0.295	0.307
10	0.358	0.353	0.401	0.387	0.241	0.260
11	0.357	0.366	0.402	0.401	0.241	0.233

Mean deviation from spectral ±0.004

Saybolt Chromometer

It was advisable to establish a correlation between this new system and the one now in general use. However, early in the work with the Saybolt instrument, it was found that that instrument did not yield comparable results for the different classes of oils and no one correlation curve could be drawn. Dependent upon other physical characteristics, each class of oils required an individual curve.

In Table V, data obtained on two series of solvents are given. These include the samples tested spectrophotometrically together with others evaluated photoelectrically. It is evident from this table that the Saybolt chromometer is primarily measuring purity, and only secondarily luminous transmission. As is indicated, the dominant wave length remains constant throughout both series. Saybolt colors and purity are plotted graphically in Figure 5. Two curves result which, even though slightly irregular in shape, illustrate that basically, purity is being measured.

Table IV. Luminous Transmission, Dominant Wave Length, and Purity

	Luminous Transmission, %		Dominant Wave Length, mμ		Purity, %	
Oil	Spectral	Color index	Spectral	Color index	Spectral	Color index
1	89.9	90.0	569	570	0.2	0.1
2	90.3	91.8	569	560	0.8	0.6
3	90.3	90.5	569	563	2.2	2.2
4	89.9	90.0	568	567	2.0	2.6
5	89.5	90.0	569	568	6.5	7.1
6	89.2	89.1	569	570	6.5	9.6
7	89.8	88.2	569	571	15.2	15.2
8	89.0	87.8	569	572	18.1	17.5
9	88.4	88.2	569	571	22.3	18.0
10	87.9	86.4	569.5	571	35.8	30.5
11	87.6	84.6	570	572	35.5	37.5
Mean deviation	±0.9%			±3 mμ		±1.5%

Purity of a color may be considered as that percentage of a pure spectral color which when mixed with C illuminant of the I. C. I. system will match the color (*14*). Since Saybolt chromometer colors are related to purity, the instrument is substantially measuring the concentration of colored bodies of the same dominant wave length. This is proved by correlating violet filter readings with "modified Saybolt" colors, which are calculated by the following equation:

$$\text{Modified Saybolt color} = \frac{(20)(\text{number of disks})}{\text{depth of oil in inches}}$$

This equation is based upon the validity of Bouguer's and Beer's laws in this range. If modified Saybolt colors of any one family of oils are plotted graphically against the logarithms of the violet filter transmissions, a linear relationship is found. Each family of oils is represented by a separate curve, but all are straight lines over the range in which these laws hold.

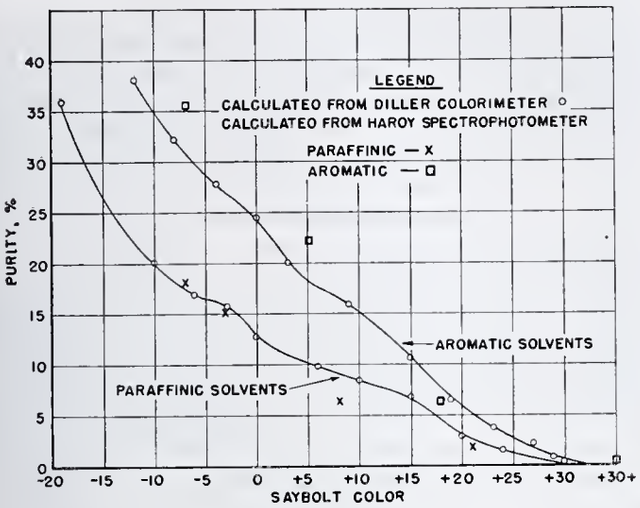

Figure 5. Relationship of Purity and Saybolt Color

Table V. Correlation of Saybolt Colors with I. C. I. Data

(Computed from Diller colorimeter readings and Hardy spectrophotometric curves)

Oil	Saybolt Color	Luminous Transmission %	Dominant Wave Length mμ	Purity %
A. Paraffinic				
1[a]	+30+	89.9	569	0.2
2	+30	90.0	579	0.2
3	+24	90.0	569	1.5
4[a]	+21	89.9	568	2.0
5	+20	89.6	569	3.1
6	+15	89.1	572	6.8
7	+10	89.1	570	8.7
8[a]	+8	89.2	569	6.5
9	+6	88.7	571	10.0
10	0	88.2	572	12.7
11[a]	−3	89.8	569	15.2
12	−3	87.8	572	15.7
13	−6	87.8	571	16.8
14[a]	−7	89.0	569	18.1
15	−10	87.3	571	20.0
16	−19	85.5	571	36.0
17[a]	Too dark	87.6	570	35.5
B. Aromatic				
1[a]	+30+	90.3	569	0.8
2	+29	90.0	566	1.0
3[a]	+27	90.3	569	2.2
4	+23	90.0	568	3.8
5	+19	90.0	568	6.5
6[a]	+18	89.5	569	6.5
7	+15	89.1	570	10.8
8	+9	88.2	571	16.0
9[a]	+5	88.4	569	22.3
10	+3	87.8	571	20.0
11	0	87.3	571	24.5
12	−4	86.9	571	27.8
13[a]	−7	87.9	569.5	35.8
14	−8	86.0	571	32.2
15	−12	85.5	571	38.0

[a] I. C. I. data obtained from Hardy spectrophotometer curves.

However, despite the apparent relationship between Saybolt color and purity, several anomalies have been encountered.

In one experiment, a solution of off-colored kerosene in carbon tetrachloride was prepared and found to match a standard disk at a height of 23.75 cm. (9.5 inches). Various amounts of water were introduced above this solution in the tube, and the color was measured again. Matches were obtained when the total heights were 9.5 inches in each case. Apparently then, even though the instrument measures color concentration, it indicated the same concentration when the colored bodies were in the ratio 9.5:8.5:7.5.

A second experiment was conducted, wherein mixtures of refined oils were prepared so that all had the same violet reading, and were closely matched visually. However, when evaluated by the Saybolt chromometer, their colors ranged from −4 to −17. Thus, even though all had the same purity, or color concentration, based upon I. C. I. data, their Saybolt colors indicated concentrations over a range directly proportional to the heights in the tubes of from 5 to 9.13 cm. (2 to 3.625 inches).

Obviously, therefore, some other factor is involved which affects the optical properties of the oils. In Table VI, other data obtained with the series of oils mentioned above are shown, which indicate that despite identical color indices, the Saybolt colors vary with surface tension, refractive index, and specific dispersion. In this particular instance, the variations are due entirely to differences in chemical composition. The oils studied here are all darker than those most frequently evaluated by the Saybolt chromometer, but were chosen primarily because they illustrate the discrepancies which may be obtained with this instrument. Similar discrepancies exist throughout the Saybolt range, as indicated by Figure 6, although they are not so pronounced.

Table VI. Saybolt Color, Refractive Index, Specific Dispersion, and Surface Tension

(All samples gave violet filter readings of 70.0)

Oil	Saybolt Color	Refractive Index	Specific Dispersion	Surface Tension Dynes/cm.
1	−4	1.3994	103	19.5
2	−6	1.4100	102	20.5
3	−9	1.4765	145	26.8
4	−13	1.4864	158	27.3
5	−17	1.5010	185	27.8

If the optical system of the Saybolt chromometer is analyzed, it becomes apparent that the physical characteristics listed in Table VI affect the results obtained. A constant source of light exists, and the oil level in one tube is so adjusted that the short-wave (violet) portion of the light energy reaching the eye is substantially equal to that passing through the standard disk and the empty tube. The energy loss in the sample tube is substantially equal to that lost because of refraction, reflection from the side walls, and absorption by the oil layer. Surface tension, in a sense, determines the contour of the meniscus which is formed at the liquid-air interface. Specific dispersion and refractive index are closely related to the energy loss at the interface, and also to the angle at which light leaves the oil surface. Thus, all three determine the average angle at which light from the interface is directed toward the side walls of the tube. The more nearly the light strikes the side walls at right angles to them, the greater will be the energy loss. Higher surface tensions, refractive indices, and specific

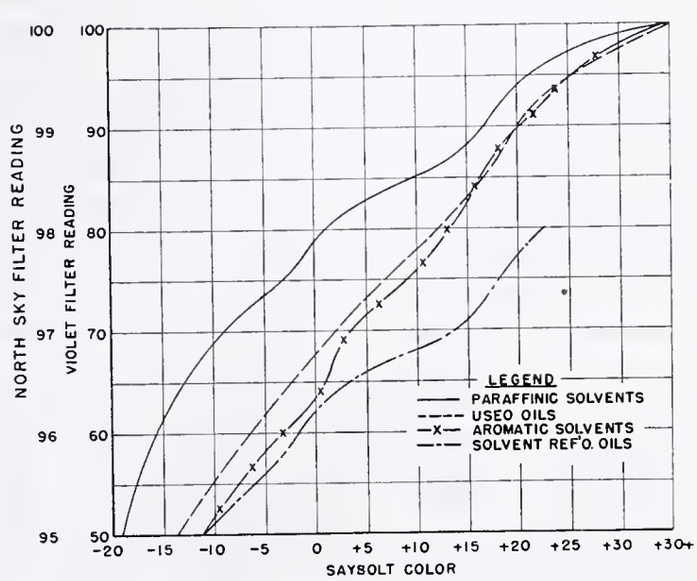

Figure 6. Conversion Chart, Color Index to Saybolt Color

dispersions individually result in greater energy losses. In order to compensate for this added loss above the oil layer, its depth must be reduced to decrease the loss due to absorption. Hence, a low Saybolt color reading results.

To illustrate the effect of surface tension, a potassium dichromate solution having a Saybolt color of +10 was prepared and measured in a sample tube of the chromometer coated with a thin film of highly viscous light-colored oil. A value of +15 was obtained, but when a few drops of wetting agent solution were added, the color was found to be +6, despite the fact that the color index was unchanged.

The personal equation involved in color measurements with the Saybolt instrument also cannot be disregarded. It has been established that there are certain individuals incapable of reproducible results. These operators frequently state that because of a different "shade" of color they are unable to match it with the standard. This is not the case with color-blind observers, or those less sensitive to minor variations in hue, who are able to obtain accurate readings even with abnormal oils.

A. S. T. M. Method D156-38 specifies the standard disks as follows:

Whole Disk	Half Disk
Y = 0.860 to 0.865	Y = 0.888 to 0.891
x = 0.342 to 0.350	x = 0.327 to 0.331
y = 0.367 to 0.378	y = 0.344 to 0.350

Using the graphs prepared by Hardy (*14*), these limits are equivalent to the following:

Whole Disk		Half Disk	
Luminous transmission, %	86.0 to 86.5	Luminous transmission, %	88.8 to 89.1
Dominant wave length, mμ	569 to 574	Dominant wave length, mμ	569 to 574
Purity, %	22.2 to 27.2	Purity, %	12.0 to 14.5

It has been shown that paraffinic and aromatic refined oils have the same dominant wave length of about 570 mμ, which is within the limits of the disks. Since the dominant wave lengths of the oil colors do not match that of the disk, they must have been varied before the light reached the eye, and that variation may be caused by differences in surface tension and refractive index. Since the energy loss is disproportionate for light of certain wave lengths, and because of the shape of the spectral curves, this results in a slight variation in dominant wave length that is easily detected by operators with keen color perception.

The effects of refractive index and surface tension are thus shown to invalidate certain Saybolt color measurements. These factors are also significant in photoelectric measurements. However, in the photoelectric colorimeter used in this work, measurements are not made through a liquid-air interface, so that surface tension has no effect. Refractive index also applies to the photoelectric measurement, but the use of a round sample tube in conjunction with the converging-diverging light beam of Diller's instrument (*8*, *10*) minimizes the refractive error, because the light enters the tube at substantially zero incidence. Provision is also made for reducing as far as possible the effect of fluorescence.

Correlation of Color Index with Saybolt Values

Since, as stated above, other physical characteristics markedly affect Saybolt values but not color index, no rigid relationship between them can be established, and each family of oils must be evaluated to fix its own correlation curve. Figure 6 shows typical correlation curves of four types of oil, prepared by evaluating paraffinic solvents, aromatic solvents, solutions of used oils in paraffinic solvents, and highly refined paraffinic spindle oils. The divergence of these curves is in accordance with the explanation already advanced.

It will be noted that all these curves and also those of Figure 5 exhibit points of inflection. These deviations from smooth curves are the results of the arbitrary table used in converting depths of oil to Saybolt colors (*5*), and the peculiar relationship between Saybolt colors and modified Saybolt colors. If these are plotted graphically, one against the other, an irregular line with the same inflection points will result. These inflections will appear in any curve obtained by plotting Saybolt values against any fundamental value such as color index or purity.

Figure 6 was prepared by evaluating over a thousand separate samples. However, similar curves can be obtained by a simpler procedure involving the linear relationship of modified Saybolt colors and the logarithms of the violet filter readings—for example, a more limited number of samples could be tested with both instruments. By computing modified Saybolt colors from the heights in the sample tube, and recording the violet transmission on the logarithmic (concentration) scale, values will be obtained through which straight lines may be drawn. These lines may then be converted to Saybolt colors and violet filter readings, yielding curves such as those shown in Figure 6. (For any given sample, instrument, and operator, deviations from these curves should be no greater than one Saybolt unit.)

Standardization and Reproducibility

Means for checking the setting of the instrument for the production of standard readings have been described (*11*). The same standard aqueous solutions were used in this study.

Since the colors of some off-test solvents and kerosenes are highly unstable, it was obviously impossible to check the reproducibility of the instrument on the samples after a period of storage. However, the colors of the series of oils listed in Table I were determined simultaneously with two instruments to demonstrate the concordance between them (Table VII). One instrument was used at the Electrical Testing Laboratories, and the second at the authors' laboratory. By means of telephone communication, it was possible to have both readings taken within 5 minutes of each other. The differences between the results are no greater than the limits of error previously established (*11*).

Table VII. Reproducibility of Results

Oil	North Sky Filter A	North Sky Filter B	Violet Filter A	Violet Filter B
1	99.5	100.5	100.0	100.0
2	102.0	102.0	101.0	101.0
3	100.5	100.5	96.0	96.0
4	100.0	100.0	94.5	95.5
5	100.0	100.0	88.0	88.0
6	99.0	99.0	84.0	84.0
7	98.0	97.5	74.5	75.5
8	97.5	97.5	72.0	72.0
9	98.0	98.0	72.0	72.0
10	96.0	96.0	56.0	56.0
11	94.0	94.0	48.0	48.0

Average deviation ±0.24 scale division.

Conclusion

The photoelectric colorimeter may be used for measuring colors of refined oils, thus providing a continuous scale for all petroleum products. This has been made possible by the use of a violet filter, which is directly related to the North Sky filter, and which has its response in the blue-violet region in which all variations in spectral absorption occur. Readings obtained with these two filters may be converted to I. C. I. data if desired.

The Saybolt chromometer, customarily used for measuring the colors of these oils, has been studied and found to be

unreliable in that erroneous and anomalous results may be obtained. Variations in physical characteristics of the oils, such as surface tension and refractive index, are probably chiefly responsible for these errors. The color index described above can be correlated with Saybolt values by the use of suitable conversion curves.

Acknowledgment

Acknowledgment is made to D. B. Judd of the National Bureau of Standards, who has carefully reviewed this paper and offered suggestions. Frequent references to a private report by V. A. Kalichevsky and B. W. Story were of great assistance in this work. The instrument used in this investigation is known as the Hellige-Diller photoelectric colorimeter, Model 405-A.

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Determination of Iodine in Tetraiodophenolphthalein

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BUTLER and Burdette (*1*) in 1939 proposed a method for determining iodine in tetraiodophenolphthalein that was much more rapid and accurate than the procedure of the U. S. Pharmacopoeia of that time. It consisted of three steps: (1) digestion with alkaline permanganate solution to decompose the material and release the iodine as iodate, (2) acidification and treatment with sodium bisulfite to reduce the manganese and iodine to their lower oxidation states, and (3) partial neutralization with ammonium carbonate and titration of the iodide with silver nitrate, using eosin or diiodofluorescein as an adsorption indicator.

Adsorption Indicator Methods

The clear advantages of the Butler-Burdette method led to its adoption by the U. S. Pharmacopoeia XII (*5*) with certain modifications. The U. S. P. XII method is almost identical with one that has been successfully used in this laboratory since October, 1941, but in which starch-iodine is used as the adsorption indicator.

If eosin or diiodofluorescein is used as adsorption indicator in the titration of the iodide, the amounts of acid and alkali added after the digestion with permanganate must be exactly such that the pH at the time of titration is between 4.5 and 7.5, preferably 6.0. On the other hand, Kolthoff's (*4*) starch-iodine complex will give sharp end points at pH's as low as 0.5.

Place 0.15 to 0.20 gram, accurately weighed, of the tetraiodophenolphthalein or its sodium salt in a 500-ml. Erlenmeyer flask, add 10 ml. of 5 per cent sodium hydroxide solution, and when the sample has dissolved on the water bath or steam bath add 25 ml. of saturated potassium permanganate solution. Digest 45 minutes on the water bath, cool, add 75 ml. of water and 10 ml. of 1 to 1 sulfuric acid, and then add 3 *M* sodium bisulfite from a buret with constant swirling of the flask until the solution is colorless. Add dilute potassium permanganate solution drop by drop till a faint permanent yellow appears. Add 10 ml. of *M* ammonium carbonate solution and 4 drops of dilute starch solution and titrate with 0.1 *N* silver nitrate solution.

Notes. The end point will be the disappearance of the last trace of blue, green, or gray, leaving the solution and the precipitate with a clear canary yellow color. If the silver iodide coagulates, the end point is best observed on the precipitate; if, as sometimes happens, it fails to coagulate, the end point is observed in the body of the solution. The end point is usually very sharp.

From 10 to 18 ml. of sodium bisulfite will be needed to reduce the iodate and permanganate. After one has observed the amount needed in a few titrations, it is preferable to add a slight excess at once from a graduate cylinder, so as to avoid the loss by volatilization of the iodine during the reduction from iodate to iodide: 15 ml. of 3 *M* sodium bisulfite would be a convenient amount.

On rare occasions, the silver iodide fails to coagulate and the starch complex gives a very vague end point.

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The procedure was checked against potassium iodide and potassium iodate, both reagent grade, and dried 1 hour at 105° C.

KI I found, 76.3 ± 0.1; calculated, 76.4%
KIO_3 I found, 59.1 ± 0.1; calculated, 59.3%

The influence of the organic material was studied by trying this procedure on mixtures of 0.1000 gram each of phenolphthalein and the potassium halogen salt.

KI I found, 76.6 ± 0.3; calculated, 76.4%
KIO_3 I found, 59.6 ± 0.4; calculated, 59.3%

A comparison of the results obtained by using starch and by using eosin as indicators in the determination of iodine in sodium tetraiodophenolphthalein is given in Table I.

Table I. Samples and Iodine Contents Found

Product No.	Weight	I (Eosin)	Weight	I (Starch)
	Gram	%	*Gram*	%
14	0.2334	53.3	0.2463	52.8
	0.2173	53.0	0.2039	52.7
			0.2074	53.0
14a	0.1978	55.5	0.2024	55.6
	0.2006	55.7	0.2100	55.4
	0.1962	55.2	0.2136	55.7
15	0.1446	52.0	0.1478	52.7
	0.1529	51.3	0.1570	52.4
	0.2051	52.5	0.2047	52.9
	0.2051	52.6	0.2040	52.6

Reduction Method

Clark and Jones (*2*) in August, 1942, published a method for determining iodine in organic compounds, based on Groak's method (*3*). Clark and Jones did not report on the applicability of their method to tetraiodophenolphthalein, but a method similar to it has been in use by the authors for this purpose since October, 1941. Their method consists of four steps: (1) boiling with alkaline permanganate to decompose the sample, if it is of an acid-insoluble alkali-soluble compound; (2) reduction of the permanganate with nitrite in acid solution; (3) destruction of the excess nitrite with sulfamic acid; and (4) titration of the iodate with potassium iodide, thiosulfate, and starch.

As used by the authors it is as follows:

Place 0.15 gram, accurately weighed, of the sample in a 500-ml. Erlenmeyer flask, add 15 ml. of 5 per cent sodium hydroxide, let it digest on the steam bath till completely dissolved, add 25 ml. of saturated potassium permanganate solution, let it digest 45 minutes, remove, add immediately 10 ml. of glacial acetic acid, and then add 5 per cent sodium nitrite solution drop by drop from a buret with rapid swirling until the red color of the permanganate has been replaced by the brown color of the manganese dioxide. Add 5 ml. of concentrated sulfuric acid slowly and with swirling to prevent loss by spattering. Once more add 5 per cent sodium nitrite solution drop by drop, and when the solution is colorless and free of manganese dioxide add 10 ml. of 5 per cent sulfamic acid solution and cool under the tap. Add 10 ml. of 33 per cent sodium hydroxide solution (5 grams of sodium hydroxide dissolved in 10 ml. of water), cool again, add 10 ml. of *M* potassium iodide solution, and titrate with 0.1 *N* thiosulfate and starch.

6 equivalents of thiosulfate =
1 mole of iodine = 127 grams of iodine

Notes. Sometimes a few particles of manganese dioxide will persist after the solution has been cleared by the sodium nitrite. Warming the solution will dissolve these; this should be done before the sulfamic acid is added.

The 5 ml. of concentrated sulfuric acid can be replaced by 10 ml. of 1 to 1 sulfuric acid.

The nitrate ion will not oxidize the iodide ion rapidly enough to interfere, if the final pH is above 2 and the solution is cool.

Occasionally the solution becomes yellow after addition of the sodium hydroxide. This usually does not affect the final result.

A less convenient but often more precise method of titration is to use 5 ml. of carbon tetrachloride instead of starch as the indicator. One must shake the solution vigorously in a stoppered Erlenmeyer flask after each addition of thiosulfate as the end point is approached. The end point, of course, is the disappearance of the pink color in the carbon tetrachloride.

Table II. Determination of Iodine

Weight of Sample	Thiosulfate Used Volume	Normality	I Found	I Calcd.
Gram	*Ml.*		%	%
0.1488	36.15	0.1000	51.4	51.2
0.1514	36.75	0.1000	51.3	
0.1505	36.50	0.1000	51.3	

Table III. Determination of Iodine

Weight of Sample	Thiosulfate Used Volume	Normality	I Found	I Calcd.
Gram	*Ml.*		%	%
KI				
0.1000	36.10	0.1000	76.35	76.4
0.1000	36.05	0.1000	76.25	
KIO_3				
0.1000	26.25	0.1064	59.1	59.3
0.1000	26.25	0.1064	59.1	
0.1000	26.25	0.1064	59.1	

The procedure was checked against *o*-iodobenzoic acid (Eastman) which melted at 161.5–162.5° C. and at 163–163.5° C. (corrected). Titration with 0.1 *N* sodium hydroxide and phenolphthalein gave an apparent equivalent weight of 247.8 ± 0.6; the calculated equivalent weight is 248.0. Because of the stability of the I—C bond in iodobenzoic acid it was necessary to replace the 45-minute digestion in alkaline permanganate by 8 hours of refluxing over a free flame (Table II).

The procedure was also checked against potassium iodide and iodate mixed with an equal weight of phenolphthalein U. S. P. (Table III). To effect complete oxidation of the phenolphthalein it was necessary to use 45 ml. of saturated permanganate and to carry out the digestion for 1 hour.

Part of the agreement between the observed and calculated values is due to a balancing of errors; if the "blank" of 0.30 ml. is subtracted, the observed values are uniformly low.

As an example of the reproducibility of this method one can consider the results of the six routine determinations made on and prior to February 23, 1943, on samples of commercial tetraiodophenolphthalein sodium of various origins (Table IV).

Table IV. Reproducibility

Sample	Weight	Thiosulfate Used Volume	Normality	I Found
	Gram	*Ml.*		%
A	0.1500	32.25	0.1064	48.4
	0.1500	32.35	0.1064	48.5
B	0.1503	35.00	0.1064	52.4
	0.1500	35.05	0.1064	52.6
C	0.0500	11.40	0.1064	51.3
	0.0500	11.30	0.1064	50.9
D	0.0500	11.30	0.1064	49.9
	0.0500	11.10	0.1064	50.9
E	0.1500	32.00	0.1064	48.0
	0.1500	31.85	0.1064	47.8
F	0.1500	33.10	0.1064	49.7
	0.1500	33.20	0.1064	49.8

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Determination of Monoalkyl Ethers of Ethylene Glycol

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WHEN toxicological work on the monoalkyl ethers of ethylene glycol was undertaken (*6*) no suitable method for determining these compounds was available. Thus an immediate need in toxicological studies as well as the likelihood of a future need in attempts to evaluate and prevent any possible hazards connected with the use of these solvents in industry led to a study of methods of estimation.

Preliminary experiments with the methyl, ethyl, *n*-propyl, and *n*-butyl derivatives, using specimens for which constants have been described (*6*), indicated that these derivatives could be oxidized quantitatively with potassium dichromate. (Methyl Cellosolve, Cellosolve, and Butyl Cellosolve are trade names for the methyl, ethyl, and butyl derivatives.) These experiments indicated, however, that progress of the oxidations was influenced by acid concentration, period of heating, order of adding reagents, and quantities of reagents used. Thus, the first part of the experimental work necessarily is concerned with the definition of conditions yielding precise quantitative reactions, while the second part is an investigation of the suitability of these reactions for estimating various concentrations in water and in air.

Experimental Method

In general, the method of conducting oxidations and determining amounts of oxygen consumed is similar to a modification of the Nicloux (*4*) method for ethyl alcohol described by Muehlberger (*3*). With the glycol ethers, however, the results were found to be most consistent when standard aqueous solutions of glycol derivatives (1, 2, or 3 ml.) were added to cooled mixtures of 5 ml. of 0.33 *N* potassium dichromate and an amount of concentrated sulfuric acid equal to the combined volume of potassium dichromate and sample solutions. This 50 per cent proportion of acid was used with all compounds. Other concentrations of sulfuric acid were investigated, but with the exception of 55 per cent acid employed in determining the propyl derivative, the other concentrations offer no advantages.

The oxidations of the aqueous samples were conducted in 25 × 200 mm. test tubes which were heated in a boiling water bath for definite periods. At the termination of heating, the tubes were cooled and the contents were transferred into 500-ml. wide-mouthed Erlenmeyer flasks. Volumes were made up to approximately 300 ml., about 3 grams of potassium iodide were added, and the liberated iodine was titrated with 0.05 *N* sodium thiosulfate. Starch solution was added, toward the end of the titration, as an indicator.

RESULTS AND DISCUSSION. Curves illustrating the effect of the period of heating on the extent of oxidations are shown in Figure 1. Each point on these curves is based on four or more determinations, and in each determination the sample (2 ml. of standard solution) was of such size that approximately a 100 per cent excess of potassium dichromate, based on complete reactions, was present.

From the four curves for oxidation with 50 per cent acid mixtures it appears that stoichiometrical reactions occur. In the order methyl, ethyl, propyl, and butyl, these reactions consumed 15.7, 13.8, 19.9, and 26 equivalents of oxygen, and they required 15, 15, 240, and 120 minutes, respectively, at water bath temperature, 90° to 100° C. As is illustrated by the curves, heating beyond these minimal periods did not significantly increase the extent of oxidation.

From the number of equivalents consumed in these reactions it appears that, in all cases, the glycol portion of the molecule is completely oxidized to carbon dioxide and water. This oxidation theoretically requires 10 oxygen equivalents. Considering the equivalents used in excess of 10 for each compound, it appears that the methyl radical is oxidized to carbon dioxide and water, the ethyl to water and acetic acid, and the propyl and butyl to carbon dioxide, water, and acetic acid. These oxidations of alkyl radicals are in agreement with the results of Polonovski (*5*) for propionic and butyric acids, Nicloux (*4*) for ethyl alcohol, and Chapman and Thorp (*1*) for the methyl radical in esters.

With an oxidizing mixture containing 55 per cent sulfuric acid, reactions requiring 20.4 instead of the theoretical 20 equivalents for the propyl derivative, occur within 60 minutes (fifth curve of Figure 1). Additional heating results in further oxidation, and at the end of 480 minutes 21.1 equivalents have been consumed. Reactions of this type are less suited to determinative work than the ones previously described; nevertheless, there are instances where a saving in time justifies the use of less accurate methods.

The theoretical considerations and results already presented suggest that theoretical factors, based on reactions requiring 16, 14, 20, and 26 equivalents, respectively, for the methyl, ethyl, propyl, and butyl derivatives, should be satisfactory for determining these glycol ethers. These factors as well as conditions producing corresponding reactions are shown in Table I.

The suitability of these factors and conditions for determining various samples in aqueous solution is indicated by recovery experiments summarized in Table II.

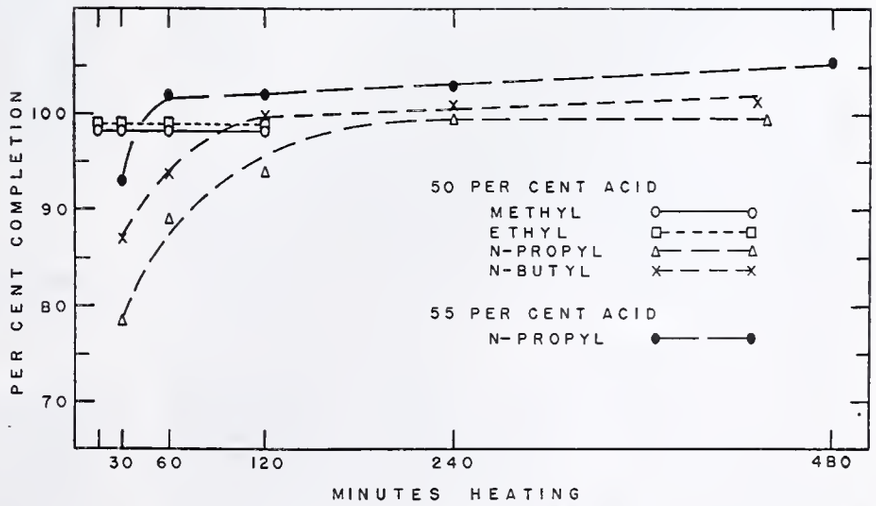

FIGURE 1. EFFECT OF PERIOD OF HEATING IN A WATER BATH ON COMPLETION OF REACTIONS

Complete reactions are theoretical ones requiring 16, 14, 20, and 26 oxygen equivalents, respectively, for the methyl, ethyl, *n*-propyl, and *n*-butyl derivatives.

Table I. Factors and Conditions Used in Determining Cellosolves

Derivative	Reaction Mixture	Heating Period	Factor, 0.33 N $K_2Cr_2O_7$
	% acid	*Min.*	
Methyl	50	30	1.584
Ethyl	50	30	2.141
n-Propyl	50	240	1.736
n-Propyl	55	60	1.736
n-Butyl	50	120	1.514

Table II. Determination of Cellosolves in Aqueous Solution

Derivative	Sample	No. of Determinations	0.33 N $K_2Cr_2O_7$ Used	Average Deviation	Recovery
	Mg.		*Ml.*	%	%
		50 Per Cent Sulfuric Acid			
Methyl	2.013	4	1.263	0.2	99.4
	4.026	4	2.491	0.1	98.2
	6.039	4	3.732	0.2	98.0
Ethyl	2.513	4	1.181	0.8	100.7
	5.026	4	2.309	0.3	98.4
	7.539	4	3.492	0.5	99.3
n-Propyl	2.005	4	1.152	0.3	100.0
	4.010	4	2.292	0.7	99.3
	6.015	4	3.338	0.5	96.3
n-Butyl	1.000	6	0.660	0.8	100.0
	2.000	6	1.318	0.7	99.8
	4.000	6	2.642	0.5	100.0
	6.000	6	3.879	1.0	97.9
		55 Per Cent Sulfuric Acid			
n-Propyl	2.005	4	1.211	0.7	104.6
	4.010	4	2.351	0.1	101.8
	6.015	4	3.374	0.3	97.4

In general, with 50 per cent acid mixtures, recoveries are not significantly affected by the size of the sample used, although there is an indication that large samples and minimal heating periods give low recoveries in determinations of the propyl derivative.

With the 55 per cent acid mixtures, recoveries of propyl glycol ether show considerable spread. They are 2.6 per cent low from large and 4.6 per cent high from small samples.

Vapors

The results presented indicated that oxidation with potassium dichromate is satisfactory for determining the glycol ethers in water solution; therefore, the suitability of the method for vapors was investigated.

Standard vapor-air mixtures were prepared by a method described by the writers (*6*). Samples were obtained in calibrated, round-bottomed flasks. Sizes of these flasks were selected for individual determinations, so that the samples collected would be equivalent to not more than 50 per cent of the potassium dichromate present. The selected samplers were charged with 5 ml. of 0.33 N potassium dichromate and 5 ml. (6 ml. in determining the propyl derivative) of concentrated sulfuric acid. They were evacuated, vapor samples were obtained, and the stoppered flasks were heated in a boiling water bath for periods listed in Table I. The flasks were cooled, and the contents transferred into 500-ml. wide-mouthed Erlenmeyer flasks for titration. The potassium dichromate consumed was determined as for aqueous samples, and the amounts of glycol ethers were found by the use of factors listed in Table I.

Results and Discussion. The agreement between average concentrations found in different chemical determinations made at intervals and concentrations calculated from weight loss, during several hours, in the saturating bubblers and total air flow used is shown in Table III. In view of the well-known difficulties in preparing and sampling standard vapor-air mixtures, the agreement shown in Table III is as good as could be expected. Apparently the reactions employed are about as suitable for determining gaseous as aqueous samples.

Elkins and co-workers (*2*) have recently described a method for the estimation of Methyl Cellosolve (ethylene glycol monomethyl ether) in the presence of certain other solvents. As a result of several determinations using 0.5 N potassium dichromate and 33 per cent sulfuric acid and gently refluxing for 4 hours, they obtained an oxygen equivalent of 15.5 for Methyl Cellosolve. In a single similar determination of Cellosolve (ethylene glycol monoethyl ether) they obtained a value of 13.2. The first equivalent is 3.1 per cent and the second is 5.7 per cent below theoretical. Thus, it appears that when these solvents alone are being determined, stronger acid solutions than those employed by Elkins can be used advantageously. With 50 per cent acid, reactions for the methyl and ethyl glycol ethers are complete within 15 minutes at boiling water bath temperature, while similar reactions require over 4 hours' refluxing with 33 per cent sulfuric acid media.

Table III. Determination of Cellosolve Vapors

Derivatives	Average Concentration Calculated	Average Concentration Found	Difference
	Mg./l.	*Mg./l.*	%
Methyl	0.93	0.88	−5.4
	2.88	2.92	+1.4
	4.03	4.12	+2.2
	5.19	5.15	−0.8
	6.59	6.54	−0.7
	6.74	6.19	−8.1
	7.03	7.16	+1.8
	10.50	9.40	−10.4
	21.08	20.36	−3.4
Ethyl	1.36	1.34	−1.5
	4.15	4.32	+4.1
	5.82	5.95	+2.2
	6.40	6.64	+3.8
	6.74	7.14	+5.9
	8.14	7.92	−2.7
	10.30	11.27	+9.4
n-Propyl	1.82	2.00	+9.9
	5.54	5.81	+4.9
	7.04	7.15	+1.6
	9.94	10.11	+1.7
n-Butyl	1.87	1.94	+3.6
	2.71	2.66	−1.8
	3.22	3.28	+1.9
	3.72	3.43	−7.8
	4.46	4.47	+0.2

Summary

Conditions for the oxidimetric determination of four monoalkyl glycol ethers have been investigated, and stoichiometrical reactions are described. In general, these reactions are but slightly influenced by heating longer than the necessary minimum in 50 per cent acid media.

The long heating period necessary for oxidation of the propyl derivative is greatly reduced by a small increase in acid concentration; however, the more rapid oxidation yields less accurate recoveries.

Acknowledgment

The writers wish to acknowledge helpful suggestions of W. F. von Oettingen of the Division of Industrial Hygiene, made in connection with this work.

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Collection and Estimation of Traces of Formaldehyde in Air

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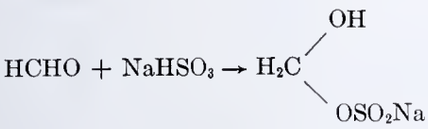
FORMALDEHYDE is one of the common contaminants of the industrial environment. Its presence in the air constitutes a potential health hazard. Maximal allowable concentrations for its presence in air continue to be formulated and this, of course, necessitates accurate methods for its estimation.

In general, small amounts of gaseous contaminants in the atmosphere are most efficiently collected for analysis by bubbling through a medium that reacts with the vapor to form a nonvolatile compound. This fixation mechanism prevents the expulsion of the gas during the sampling by the aerating action of the unabsorbed constituents. As is well known, formaldehyde gas reacts rapidly with aqueous solutions of sodium bisulfite to form the nonvolatile sodium formaldehyde–bisulfite compound:



This derivative is stable in slightly acid and neutral solutions and can be decomposed only when the solution is made distinctly alkaline. These properties permit the direct estimation of small amounts of formaldehyde by destroying excess bisulfite with iodine at pH 6 to 7, and subsequently liberating the sulfite combined as sulfoxylate by proper adjustment of the hydrogen-ion concentration. The direct titration of this dissociated bisulfite with a standard iodine solution affords an accurate measure of the formaldehyde collected, which is independent of change in the bisulfite-ion concentration caused by atmospheric oxidation of the collection medium.

Analytical methods for different aldehydes based on the formation of sulfoxylate have been described by Clausen (*3*), Donnally (*4*), Edwards (*5*), and Friedemann (*6*). These methods were devised chiefly for the determination of the acetaldehyde-bisulfite derivative formed in the assay of lactic acid. The principal difficulty in the titration of the formaldehyde sulfoxylate is its slow rate of decomposition in slightly alkaline solutions. While readily decomposed by caustic solutions, the latter interfere with the iodometric determination of the liberated sulfite. Investigation revealed that the formaldehyde complex could be hydrolyzed instantaneously at room temperature by the addition of a solution of sodium carbonate and sodium acetate, and that the resulting solution could be titrated to a stable stoichiometric end point.

The formation of bisulfite addition compounds is a characteristic reaction of aldehydes and ketones. A method based on this reaction is not subject to interference by sulfur dioxide, alcohol, the halogen gases, and volatile organic acids which interfere with the determination of formaldehyde using Schiff's reagent (*1*). The method, as proposed, assays total aldehyde vapor. In the examination of plant atmospheres for toxic constituents, the identity of the vapors is usually available to the analyst from knowledge of the manufacturing process.

On rare occasions where the formaldehyde is accompanied by acetone, a separation can be effected by means of sodium bicarbonate. The acetone derivative is completely decomposed in media containing a low concentration of sodium bicarbonate, whereas the formaldehyde-bisulfite is dissociated only when the solution is made alkaline with sodium carbonate.

Method of Analysis

REAGENTS. 1 per cent sodium bisulfite, $NaHSO_3$.

0.1 *N* iodine solution. This reagent need not be standardized.

0.01 *N* iodine, prepared by dilution of 0.1 *N* iodine solution. This solution must be standardized daily by titration against 0.05 *N* sodium thiosulfate.

1 per cent starch solution.

Sodium carbonate buffer prepared by dissolving 80 grams of sodium carbonate in about 500 ml. of water, adding 20 ml. of glacial acetic acid, and diluting to 1 liter.

METHOD. The air is drawn through a midget impinger (*8*) containing 10 ml. of 1 per cent sodium bisulfite solution at a rate of 1 to 3 liters per minute. At the termination of the sampling the bisulfite solution and washings are transferred to a 300-ml. cone flask. When sampling at 28 liters per minute the large impinger (*8*) is filled with 100 ml. of bisulfite solution and a 10-ml. aliquot is removed for analysis. The solution is titrated with the 0.1 *N* iodine solution to a dark blue end point, using 1 ml. of starch as an indicator. Excess iodine is destroyed by the addition of 1 or 2 drops of 0.05 *N* sodium thiosulfate, and the solution is equilibrated to a faint blue color by the dropwise addition of the 0.01 *N* iodine solution. The excess inorganic bisulfite is thereby completely oxidized to sulfate, and the solution is now ready for assay of the formaldehyde-bisulfite compound.

Twenty-five milliliters of the sodium carbonate reagent are added and the liberated sulfite is titrated with 0.01 *N* iodine solution to a faint blue end point. If acetone is present, the solution is treated with 2 ml. of 5 per cent sodium bicarbonate after the destruction of the free inorganic sulfite in the collection medium. The sulfite liberated by the bicarbonate is removed by addition of 0.01 *N* iodine solution and the pH is finally adjusted by means of the sodium carbonate solution for the final titration of the formaldehyde-bisulfite. When the volume of solution used for the titration is less than 1 ml. it is desirable to run a blank on 10 ml. of 1 per cent sodium bisulfite. This blank correction does not exceed 0.10 ml. and is negligible in most assays. One milliliter of 0.01 *N* iodine solution is equivalent to 0.15 mg. of formaldehyde.

The accuracy of the method was tested by adding measured volumes of a standardized formaldehyde solution to 10-ml. portions of 1 per cent sodium sulfite and analyzing the mixture by the described procedure. The standard formaldehyde solution prepared by diluting the 37 per cent commercial solution to a concentration of about 1 mg. per ml. was assayed by the method of Romijn (*7*). The results shown in Table I exhibit good agreement between the formaldehyde taken and recovered.

By using a microburet reading to 0.01 ml. and delivering droplets of about 0.03 ml. it is possible to establish the two end points with a maximum error of 0.06 ml. and this introduces a possible error of 1 per cent in the volumetric estimation of 1 mg. of formaldehyde. With smaller quantities of formaldehyde, the percentage error increases rapidly, and it is desirable to sample a sufficient volume of air to provide at least 0.5 mg. of formaldehyde for the analysis. In the case of atmospheres that contain formaldehyde concentrations of about 20 parts per million, approximately 20 liters of air must be sampled for each 10 ml. of bisulfite solution in the impinger.

The formation of the formaldehyde-bisulfite is not interfered with by the concomitant presence of small amounts of methyl alcohol, bromine, or acetic acid, which may on occasion be collected in the sodium bisulfite solution. This was tested by adding the substances shown in Table II to 10 ml. of 1 per cent sodium bisulfite containing 1.03 mg. of

Table I. Analyses of Formaldehyde Solutions

Formaldehyde Taken	Formaldehyde Recovered	Difference
Mg.	*Mg.*	*Mg.*
0.021	0.024	+0.003
0.053	0.058	+0.005
0.106	0.110	+0.004
0.216	0.220	+0.004
0.426	0.432	+0.006
0.638	0.625	−0.013
1.064	1.085	+0.021
1.276	1.270	−0.006
1.49	1.46	−0.03
2.13	2.04	−0.09
3.19	3.01	−0.18
5.32	5.24	−0.08

Table II. Effect of Other Vapors on Estimation of Formaldehyde

Formaldehyde Taken	Foreign Vapor		Formaldehyde Recovered
Mg.	*Mg.*		*Mg.*
1.03		None	1.02
1.03		None	1.04
1.03	20	Methyl alcohol	1.05
1.03	50	Acetic acid	1.05
1.03	10	Bromine	1.02
1.03	50	Bromine	1.00
1.03	10	Acetone[a]	1.01

[a] Acetone-bisulfite dissociated by means of sodium bicarbonate, prior to hydrolysis of formaldehyde sulfoxylate.

formaldehyde and analyzing the mixtures by the proposed method.

In the absence of other aldehydes the use of bisulfite for the collection medium affords a simple and rapid method for the estimation of the formaldehyde.

Efficiency of Bisulfite as a Collection Medium

The retentive power of sodium bisulfite was studied by blowing air through dilute solutions of formaldehyde and passing the vapors, freed from spray by means of a glass wool plug, through a train of two impingers. Both impingers contained sodium bisulfite, and in each case a similar experiment was conducted in which the vapor was first bubbled through water and then through a second impinger containing 1 per cent sodium bisulfite solution. After analysis of the four solutions it was possible to compare the relative collecting efficiencies of water and the bisulfite solution under similar conditions of air flow and formaldehyde vapor concentration. Preliminary tests in which known amounts of dilute formaldehyde solutions were completely volatilized by a stream of dry warm air and collected in bisulfite revealed that the vapor was recovered quantitatively, and that at a flow of 3 liters per minute, 97 per cent was caught in the first impinger.

On the basis of these experiments, it is safe to calculate the efficiency as the ratio of the weight of formaldehyde collected in the first impinger to the total weight found in the train of collecting tubes. Reproducible rates of air flow were secured in the case of the slow speed measurements with the aid of a large Mariotte flask. It was observed that formaldehyde vapor was strongly absorbed by rubber tubing. To avoid loss of formaldehyde from this source, impingers were assembled for the testing work that have the entrance and exit tubes in a straight line with abutting joints. This permits glass-to-glass contact between adjoining impingers, using little or no exposed rubber surface.

The results of these experiments (Table III) show that at slow rates of air flow (1 to 3 liters per minute) about 98 per cent of the formaldehyde is collected in a single tube of bisulfite solution, and that even at a flow of 28 liters per minute the 95 per cent recovery makes the use of a single impinger tube permissible.

Under the same conditions water retains between 69 and 87 per cent of the formaldehyde vapor. The results also reveal that the collection efficiency increases with increase in the formaldehyde vapor concentration. The 95 per cent absorption in 1 per cent sodium bisulfite is in good agreement with the value of 93.6 per cent reported by Barnes and Speicher (*2*) using 1.25 per cent potassium hydroxide as the collection medium, and the same air flow of 28 liters per minute.

Summary

Formaldehyde vapors can be collected quantitatively in a single impinger tube containing 1 per cent sodium bisulfite. The resultant solution is analyzed volumetrically by titrating the formaldehyde-bisulfite by means of a standard iodine solution. The simplicity of the procedure provides a convenient method for field work.

Table III. Comparative Efficiency of Sodium Bisulfite and Water as Media for the Collection of Formaldehyde

Air Flow	Formaldehyde Concentration	Collected by 1st Bubbler 1% $NaHSO_3$	Water
L./min.	*P. p. m.*	%	%
1	7	100	77
1	21	97	79
1	101	98	87
3	7	96	68
3	20	97	74
3	78	98	81
28	7	95	69
28	14	95	72
28	41	95	76

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Furfural

(Correspondence)

Sir: The article on furfural by Ira J. Duncan [Ind. Eng. Chem., Anal. Ed., 15, 162 (1943)] brought to mind some work I once did on preparation of furfural from ground corncobs. I also observed the decomposition of furfural by hot dilute acid and reasoned that considerable furfural is probably destroyed during the hydrolysis of the pentosans before it is liberated from the hot acid. If the concentration of furfural in the acid can be reduced, yield should increase.

One way to accomplish a reduction in furfural concentration in the reaction mixture is by the addition of sodium chloride. When large quantities of salt were added to the acid, it was found that the yield of furfural could be increased from about 10 per cent to about 15 per cent of the weight of the cobs, indicating a considerable destruction of furfural in ordinary hydrolysis. Perhaps some use could be made of this technique in analytical determinations.

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Determination of Small Amounts of Tellurium in High-Lead and Tin-Base Alloys

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A simple, rapid, and accurate volumetric method for the quantitative determination of tellurium in high-lead and tin-base babbitts has been developed. The analytical details for quantitative determination of small quantities of tellurium are given.

IN THE course of an extensive research program on the improvement of tin- and lead-base babbitts for bearing purposes, the addition of tellurium was studied thoroughly. The analytical determination of these tellurium additions was very important. The various analytical methods recorded in the literature were tried and found to be either too long and tedious or of insufficient accuracy (*1*, *3–9*).

The method developed in this laboratory is based upon dissolving the alloy in concentrated hydrochloric acid with the aid of several grams of potassium chlorate, diluting the solution with water to approximately an acid concentration of 10 per cent by volume, and precipitating the tellurium from solution by saturating with sulfur dioxide. The solution is filtered and the precipitate is dissolved in dilute nitric acid (2 to 1), which oxidizes the tellurium only to the valence of 4 (*2*). Potassium iodide is added in excess and the liberated iodine is titrated with sodium thiosulfate.

The following equations are involved:

$$H_2TeO_3 + 4KI + 4HNO_3 = Te + 2I_2 + 4KNO_3 + 3H_2O$$

$$I_2 + 2Na_2S_2O_3 = 2NaI + Na_2S_4O_6$$

Procedure

TELLURIUM-BEARING TIN-BASE ALLOYS. Weigh a 5.00-gram sample of the tin-base alloy containing the tellurium into a 400-ml. beaker. Add approximately 5 grams of potassium chlorate and 50 ml. of concentrated hydrochloric acid (22° Bé.) and heat to the boiling point; if the solution is not complete add more potassium chlorate and repeat the process.

For every volume of solution add 2 volumes of water and boil for at least 5 minutes. Add 10 ml. of a 1 per cent aqueous solution of hydrazine sulfate to the sample containing the tellurium and heat to the boiling point. Precipitate the tellurium by bubbling the sulfur dioxide through this hot solution for at least 15 minutes. After precipitation is complete allow to settle for at least one hour, filter on a platinum cone with suction, using Whatman 41H filter paper or a similar grade of paper and wash the tellurium with hot water until the chloride ions are completely removed. Dissolve the precipitate from the filter paper with successive portions of warm dilute nitric acid (2 to 1), to the extent of 25 ml. Wash and dilute the filtrate to approximately 200 ml.

Add about 2 grams of urea to eliminate any small amount of nitrous acid which may be present, cool to room temperature, and add potassium iodide in excess. After 1 minute titrate the liberated iodine with sodium thiosulfate solution, using starch as an indicator. The end point is permanent for several minutes.

In alloys containing less than 1 per cent of tellurium the use of 0.01 *N* sodium thiosulfate gives an accuracy of 0.02 per cent. However, for higher percentages of tellurium satisfactory results can be obtained with more concentrated sodium thiosulfate solutions.

TELLURIUM-BEARING LEAD-BASE ALLOYS. Weigh a 5.00-gram sample of the lead-base alloy containing tellurium into a 500-ml. flask, add 30 ml. of concentrated sulfuric acid (66° Bé.) and 10 grams of potassium hydrogen sulfate, and heat until all the metal is dissolved and the precipitated salts appear white. Allow to cool, add 80 ml. of water and 10 ml. of concentrated hydrochloric acid, stir the solution well to dissolve the tellurium completely, filter into a 400-ml. beaker, and wash. Add 80 ml. of concentrated hydrochloric acid, dilute the solution to 300 ml. with water, and pass a rapid stream of sulfur dioxide through the hot solution; then proceed as in the case of tin-base alloys.

Discussion

In order to check the accuracy of this method chemically pure tellurium dioxide was purchased from Eimer and Amend. With this material as a source of the tellurium the following data were obtained by using aliquot parts:

Tellurium Taken	Tellurium Found	
	Titration method	Gravimetric method
Gram	*Gram*	*Gram*
0.0080	0.0080	0.0081
0.0112	0.0112	0.0114
0.0160	0.0159	0.0162
0.0189	0.0189	0.0191

The addition of various amounts of metallic tellurium to standard alloys also gave very satisfactory results with the titration method. Gravimetric results show a greater deviation, probably due to contamination. These samples were taken in the same manner as in the case of the pure tellurium dioxide.

The following table shows the results when various amounts of tellurium were added to a standard tin-base babbitt, alloy No. 54a, purchased from the National Bureau of Standards:

Tellurium Taken	Tellurium Found	
	Titration method	Gravimetric method
Gram	*Gram*	*Gram*
0.0100	0.0101	0.0103
0.0186	0.0184	0.0190
0.0216	0.0216	0.0221
0.0268	0.0268	0.0273

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Mixed Solvent Extraction

Notes on an Analytical Method

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Hunter recently presented a graphical method of attack for mixed solvent extraction problems involving four components. The equilateral tetrahedron used by Hunter is modified in this article to a rectangular type of tetrahedron, three of whose edges coincide with the three axes of a rectangular coordinate system, and three of whose faces are isosceles right triangles. With rectangular coordinates, the methods of analytical geometry are readily applied. The problem given by Hunter is solved analytically and the solution presented in the form of generalized equations in terms of values obtainable from the characteristics of the system and from the statement of the problem. The numerical example given by Hunter is solved by this method. The results compare well with the graphical method, and there is a large saving in the time required for solution of the problem.

A RECENT article by Hunter (*1*) presented a graphical method of computation for four-component solvent extraction problems. This article shows the application of analytical geometry methods to such a problem, with large savings in the time and the difficulty of solution.

Four-Component System on Rectangular Coordinates

The system of representation used by Hunter consisted of a regular equilateral tetrahedron, but such a system does not lend itself readily to the use of analytical geometry methods. Kinney (*3*) pointed out that the equilateral method of representation for three components can easily be converted to a right-triangle system, using the ordinary x–y coordinates. In such a case, the third component is defined by the other two, since the total of the weight fractions must equal unity. This method is easily extended to four components, using the ordinary x–y–z coordinates of analytical geometry. In this case, three edges of the tetrahedron coincide with the three axes, and x, y, and z become the weight fractions of three of the components. The fourth component is thus defined, since the total of the weight fractions of the four components must equal unity.

Figure 1 shows the problem given by Hunter for the system acetone–chloroform–water–acetic acid, where x, y, and z represent the weight fractions of water, acetic acid, and acetone, respectively. For convenience in comparison with the Hunter article, the apexes of the tetrahedron are labeled A, B, S_1, and S_2. The line RT is that given by Hunter and is the intersection of two planes, each of which is determined by the tie line in one face and the opposite apex.

The plane containing the tie line in the x–y plane and the z apex has the equation

$$\frac{x}{k_1} + \frac{y}{a} + \frac{z}{1} = 1 \tag{1}$$

Similarly, the plane containing the tie line in the x–z plane and the y apex is represented by Equation 2.

$$\frac{x}{k_2} + \frac{y}{1} + \frac{z}{c} = 1 \tag{2}$$

Equations 1 and 2 together define the line of intersection, RT. It is desired to find the intersection of line RT with the straight line joining corresponding points on the two equilibrium curves. Except for the case of RT parallel to the x axis, the intersection can be found by intersecting plane

$$\frac{y}{y^*} + \frac{z}{z^*} = 1 \tag{3}$$

with the planes defined by Equations 1 and 2. For the final solution, corresponding values of x, y^*, and z^* can then be obtained from the equilibrium data of the systems involved.

Equations 1, 2, and 3 are solved simultaneously by first eliminating x between Equations 1 and 2, then solving the result with Equation 3 to obtain z, giving

$$z = \frac{(k_2 - k_1) - (k_2 - k_1/a)y^*}{\left(\frac{k_2}{c} - k_1\right) - (k_2 - k_1/a)y^*/z^*} \tag{4}$$

This value of z is then placed in Equation 3 and the equation solved for y, giving

$$y = \frac{\left(\frac{k_2}{c} - k_1\right) y^* - (k_2 - k_1)y^*/z^*}{\left(\frac{k_2}{c} - k_1\right) - (k_2 - k_1/a)y^*/z^*} \tag{5}$$

These values for y and z are then placed in Equation 2 and the equation is solved for x, giving

$$\frac{x}{-k_1k_2} = \frac{(1/ca - 1)y^* - (1/c - 1) - (1/a - 1)y^*/z^*}{(k_2/c - k_1) - (k_2 - k_1/a)y^*/z^*} \tag{6}$$

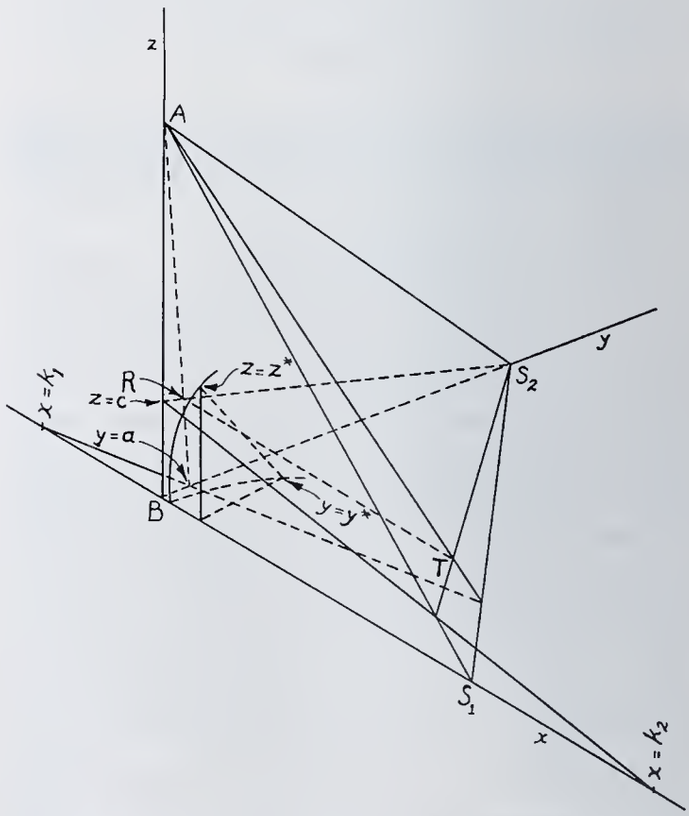

FIGURE 1. FOUR-COMPONENT SYSTEM REPRESENTED ON RECTANGULAR COORDINATES

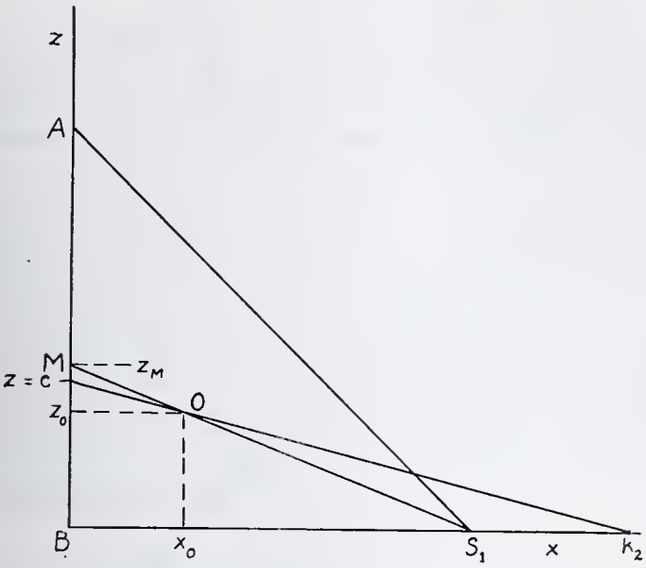

FIGURE 2. RELATION OF TIE LINE LOCATION IN x–z PLANE TO RELATIVE AMOUNTS OF MIXTURE AND SOLVENT

Knowledge of y^*, z^*, k_1, k_2, a, and c allows solution of Equations 4, 5, and 6. Tarasenkov (*6*) has pointed out that the tie lines of many systems intersect at a point on the extension of a side of the phase triangle. For such a case,

$$\frac{y^*_1}{k_1 - x_1} = \frac{y^*_2}{k_1 - x_2} \tag{7}$$

$$\frac{z^*_1}{k_2 - x_1} = \frac{z^*_2}{k_2 - x_2} \tag{8}$$

where subscripts 1 and 2 on x, y, and z refer to the two layers, the B-rich and the S_1-rich, respectively. These equations represent a type of distribution equation which Tarasenkov believes has advantages over other types proposed. Othmer and Tobias (*5*) suggest a distribution equation of the form

$$\log \frac{1 - b_1}{b_1} = n \log \frac{1 - x_2}{x_2} + C \tag{9}$$

where b is the weight fraction of B, and n and C are constants.

One readily apparent advantage of Equations 7 and 8 over Equation 9 is that no plotting is necessary. Each set of equilibrium values determines a value of k, either k_1 or k_2. The several values of k obtained may then be averaged and statistical calculations applied to eliminate poor observations.

The intersection of tie lines in a point may not be a universal rule for all such systems. The author believes, however, that this property of intersection of tie lines is characteristic of a normal type of distribution for liquid-liquid systems, analogous to the Raoult law for liquid-vapor equilibria.

The values of a and c can be directly related to the compositions and the amounts of the mixture and the solvent. Figure 2 shows the x–z plane of the tetrahedron.

Now

$$\frac{\text{wt. of } S_1 \text{ in } S}{\text{wt. of } M} = \frac{\text{wt. of } S}{\text{wt. of } M} \times \frac{\text{wt. of } S_1}{\text{wt. of } S} = R_M x_S \tag{10}$$

and from Figure 2, by geometry,

$$\frac{\text{wt. of } S_1 \text{ in } S}{\text{wt. of } M} = \frac{OM}{OS_1} = \frac{x_0}{1 - x_0} \tag{11}$$

or

$$\frac{x_0}{1 - x_0} = R_M x_S \,. \tag{12}$$

and

$$x_0 = \frac{R_M x_S}{R_M x_S + 1} \tag{13}$$

Also

$$\frac{OM}{OS_1} = \frac{z_M - z_0}{z_0} \tag{14}$$

so that

$$z_0 = \frac{z_M}{R_M x_S + 1} \tag{15}$$

From the similarity of right triangles in Figure 2,

$$\frac{c}{k_2} = \frac{z_0}{k_2 - x_0} \tag{16}$$

Combining Equations 13, 15, and 16 and simplifying, gives

$$c = \frac{z_M k_2}{R_M x_S (k_2 - 1) + k_2} \tag{17}$$

Similarly

$$\frac{\text{wt. of } S}{\text{wt. of } B} = \frac{\text{wt. of } S}{\text{wt. of } M} \times \frac{\text{wt. of } M}{\text{wt. of } B} = \frac{R_M}{1 - z_M} \tag{18}$$

and from Figure 3,

$$\frac{\text{wt. of } S}{\text{wt. of } B} = \frac{OB}{OS} = \frac{x_0}{x_S - x_0} \tag{19}$$

so

$$x_0 = \frac{R_M x_S}{R_M + 1 - z_M} \tag{20}$$

Also from Figure 3,

$$\frac{OB}{OS} = \frac{y_0}{y_S - y_0} \tag{21}$$

so

$$y_0 = \frac{R_M y_S}{R_M + 1 - z_M} \tag{22}$$

or, since $y_S + x_S = 1$

$$y_0 = \frac{R_M (1 - x_S)}{R_M + 1 - z_M} \tag{23}$$

Then from the similarity of right triangles in Figure 3,

$$\frac{a}{-k_1} = \frac{y_0}{x_0 - k_1} \tag{24}$$

Combining Equations 20, 23, and 24 gives

$$a = \frac{R_M (1 - x_S)}{R_M + 1 - z_M - R_M x_S / k_1} \tag{25}$$

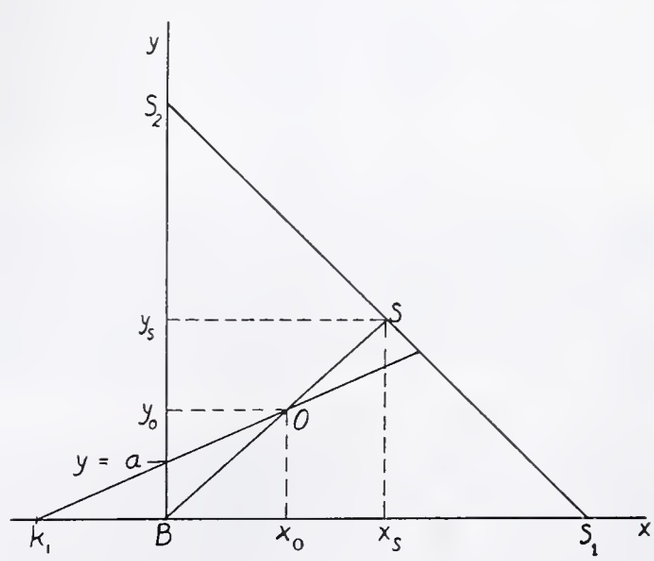

FIGURE 3. RELATION OF TIE LINE LOCATION IN x–y PLANE TO RELATIVE AMOUNTS OF MIXTURE AND SOLVENT

For systems whose tie lines do not intersect in a point on the base line, the particular tie lines specified by the problem in question can be used to determine the values of k_1 and k_2 for solution of the equations. The solution is also valid for k_1 and k_2 both positive, both negative, or one negative and the other positive.

Table I. Comparison of Values Obtained by the Several Methods

Phase	x	y	z
1. Upper layer			
Experimental	0.574	0.316	0.075
Graphical	0.578	0.318	0.066
Analytical	0.565	0.323	0.069
2. Lower layer			
Experimental	0.028	0.096	0.203
Graphical	0.033	0.100	0.202
Analytical	0.024	0.095	0.207

Example

Taking the example used by Hunter (*1*): A mixture, consisting of 27.8 per cent by weight of acetone and 72.2 per cent of chloroform is to be batch-extracted at 25° C. in a single stage with a mixed solvent, consisting of 58.5 per cent of water and 41.5 per cent of acetic acid. The ratio of the weight of mixed solvent to the weight of treated mixture is to be 0.93. Then R_M is 0.93, x_S is 0.585, and z_M is 0.278.

From the experimental data given by Hunter, three values of k_1 may be calculated from Equation 7: −0.239, −0.273, and −0.264, with a mean of −0.259. Three values of k_2 may be calculated from Equation 8: 1.44, 1.56, and 2.04, with a mean of 1.68. The third of these values of k_2 differs from the mean by 3.57 times the standard deviation for the three values. On a plot of Equation 9, for the data from which these three values of k_2 were calculated, the data giving the value of k_2 as 2.04 gave a point differing widely from other determinations on this system (*5*), while the two groups giving k_2 values of 1.44 and 1.56 gave good agreement. For these reasons, the value of 2.04 for k_2 has been discarded, and a mean of 1.50 used in this example for k_2.

Substituting these values in Equation 25 gives

$$a = \frac{0.93(1 - 0.585)}{0.93 - 1 - 0.278 + 0.93(0.585/0.259)} = 0.103$$

and

$$c = \frac{0.278 \times 1.50}{0.93(1.50 - 1)0.585 + 1.50} = 0.235$$

Substituting the known and calculated quantities in Equations 4, 5, and 6 gives

$$x = \frac{15.7y^* - 3.40y^*/z^* - 1.27}{6.64 - 4.02y^*/z^*} \quad (26)$$

$$y = \frac{6.64y^* - 1.759y^*/z^*}{6.64 - 4.02y^*/z^*} \quad (27)$$

$$z = \frac{1.759 - 4.02y^*}{6.64 - 4.02y^*/z^*} \quad (28)$$

Figure 4 shows data plotted from Hunter (*1*) for the systems water–acetic acid–chloroform and water–acetone–chloroform. The values of y^* versus x shown in Figure 4 are for the first of these systems. and the values of z^* versus x are for the latter. Values of the ratio y^*/z^* are also plotted versus x in Figure 4 for each of these systems to aid in the calculations.

Equation 26 is solved as follows:

A value of x is assumed, the corresponding values of y^* and y^*/z^* are read from Figure 4, and these values are used to calculate x from Equation 26. This process is repeated until the calculated value of x equals the assumed value. The value of x so obtained is used to find the corresponding values of y^* and y^*/z^* from Figure 4 to use in solving Equations 27 and 28 for y and z. This process is repeated for the other layer.

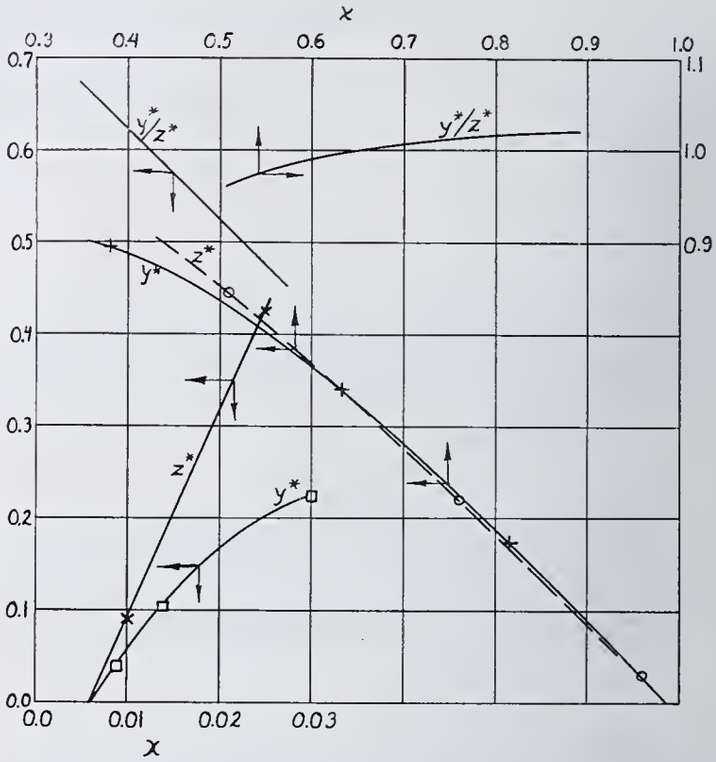

Figure 4. Equilibrium Relationships for Illustrative Problem from Data of Hunter (*1*) for System Water–Acetone–Acetic Acid–Chloroform

Table I shows a comparison of values calculated by this method with both the graphical and experimental values of Hunter (*1*). The comparison is satisfactory.

Solvent Refining of Oils

The method described above for four-component systems is suitable for use in calculations on the solvent refining of oils by two immiscible solvents, provided that the assumptions of Hunter (*1*) apply. These assumptions are (1) that the line of intersection, RT, in Figure 1 is the desired tie line and (2) that the boundary line running from $z = z^*$ to $y = y^*$ is a straight line. A test of these assumptions is to calculate the compositions on the basis that the assumptions are valid, and then check against the experimental results.

Two changes in the method are necessary for solvent-refining calculations. The first is to change the x and y axes from weight fractions to volume fractions of the solvents. This also requires all calculations to be on the basis of volumes rather than weights. The second change is to place an auxiliary scale on the z axis graduated in terms of the physical property, such as density, refractive index, or viscosity gravity constant, which is used to characterize the stock to be treated. This method of plotting has been described by Hunter and Nash (*2*) and by Kurtz (*4*). Values of this property as a function of volume fraction of solvent S_1 are plotted in the x–z plane. The scale used for the physical property should be such as to give a suitably sized equilibrium curve (*2*). Once plotted, values of z^* for use in the calculations should not be read from the property scale, but rather from the normal z scale which is graduated from 0 to 1. From this point on, the calculations are identical with those described above for four-component systems.

Conclusions

The methods of analytical geometry have been applied to the solution of four-component system problems with

satisfactory results. To facilitate use of the method, the equilateral tetrahedron has been distorted to a tetrahedron of three isosceles right-angle triangular faces, which tetrahedron is placed on an x–y–z rectangular coordinate system. Equations have been derived in terms of general properties of liquid-liquid equilibrium systems. The introduction of the use of the intersection point of the tie lines on the extension of the base line of the phase triangle allows generalized equations to be derived for the solution of varied problems on the same system. Additional equations have been derived which relate the above equations to the conditions of a specific problem. The resultant saving in time by this analytical method is large over the graphical method of Hunter (*1*), especially if a number of problems must be worked on the same system. The accuracy of the analytical method has been shown to be satisfactory. Its application to solvent refining has also been shown.

Nomenclature

A, B = components of treated mixture
C = constant, Equation 9
M = treated mixture
R_M = ratio of weight of mixed solvent to weight of treated mixture
S = mixed solvent
S_1, S_2 = components of mixed solvent
a = intercept of tie line in the x–y plane with y axis
b = weight fraction of component B in the treated mixture
c = intercept of tie line in the x–z plane with the z axis
k = intercept of tie line on x axis
n = constant, Equation 9
x = weight fraction of component S_1
x_S = weight fraction of component S_1 in mixed solvent S
y = weight fraction of component S_2
y_S = weight fraction of component S_2 in mixed solvent S
y^* = value of y from the equilibrium curve in the x–y plane at a given value of x
z = weight fraction of component A
z_M = weight fraction of component A in treated mixture M
z^* = value of z from the equilibrium curve in the x–z plane at a given value of x

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Determination of Halogens in Organic Compounds

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THE decomposition of organic halogen compounds by the sodium-alcohol method (*12*) has advantages over most other procedures in the simplicity of both the technique and apparatus required. However, the usefulness of the method has suffered not only from inherent limitations, such as the inability to decompose effectively gaseous or very volatile compounds, but also from some degree of unreliability. Uncertainties in the analyses have been experienced mostly with the aromatic compounds in which the halogens are firmly bound. This unreliability is evident from the number of papers that have appeared on this method of analysis, and which list in detail the conditions that are necessary for good results (*1*, *2*, *3*).

A number of modifications have been tried in order to improve the reliability of the original procedure. These include increasing the amount of sodium used (*1*, *13*), and substituting higher boiling alcohols for the ethyl alcohol. The alcohols that have been used are butyl, amyl (*9*), isoamyl (*4*), and benzyl (*6*). Mixtures of a high boiling, inert solvent and an alcohol, such as kerosene and amyl alcohol (*8*), have been tried. With the exception of butyl alcohol, the use of these higher alcohols has the disadvantage of requiring a water extraction of the halide ion before its determination. Furthermore, these alcohols have only slightly greater usefulness than ethyl alcohol, as results which have been reported show the difficulty in obtaining complete decomposition of some of the compounds analyzed. Monoethanolamine-dioxane mixtures have been successfully used as a solvent in this method of analysis (*10*).

Table I. Analysis of Halogen Compounds

Compound	Halogen Found: Isopropyl alcohol	Halogen Found: *sec*-Butyl alcohol	Halogen Calculated
	%	%	%
1,2,4,5-Tetrachlorobenzene	65.6	65.7	65.7
Hexachlorobenzene	74.7	74.7	74.7
Hexachlorodiphenyl[a]	60.3	60.3	59.0
	60.2	60.2	
Heptachlorodiphenyl oxide[a]	60.3	60.2	60.3
	60.3	60.2	
Bromobenzene	50.9	51.0	50.9
o-Fluorodiphenyl	10.7	10.8	11.0
		11.0	

[a] Commercial mixtures.

Improvement in Method

Secondary alcohols, notably isopropyl and *sec*-butyl alcohol, have been found to be particularly effective solvents for the decomposition of halogen compounds with sodium. Isopropyl alcohol is soluble in water and *sec*-butyl alcohol is sufficiently soluble so that extraction of the halide ion is not necessary before its determination.

All the chlorine and bromine compounds, which have been analyzed with these solvents, have been easily and completely decomposed. Results for a few of the more refractory type compounds are given in Table I. The applicability of this method to the analysis of chlorine compounds, and also to the less stable bromine and iodine compounds, is further indicated by the favorable results obtained in the analysis of an aromatic fluorine compound, *o*-fluorodiphenyl. In this

case, a longer period of heating was necessary to secure complete decomposition.

Discussion

Some of the advantages which isopropyl and *sec*-butyl alcohol have over primary alcohols in this method of analysis are due to their slower rates of reaction with sodium. When ethyl alcohol is used as the solvent, the evolved hydrogen covers the surface of the sodium and prevents effective decomposition of the halogen compound (*8*). This kind of interference is considerably reduced with the secondary alcohols. Also, the slow rate of formation of the sodium secondary alcoholates is a further advantage, as no interfering precipitates are formed during the decomposition. The precipitation of sodium ethoxide from ethyl alcohol is detrimental to the analysis of insoluble compounds.

sec-Butyl alcohol has been refluxed from 5 to 6 hours with sodium without the precipitation of sodium secondary butoxide. Isopropyl alcohol starts to precipitate sodium isopropoxide in 3 to 4 hours, depending somewhat on the conditions. As all the chlorine and bromine compounds, which have been analyzed, were completely decomposed in isopropyl alcohol before the appearance of sodium isopropoxide, this would not be a factor in the analysis of these compounds. For this reason, *sec*-butyl alcohol would be the preferred solvent for compounds that may require periods of decomposition longer than 4 to 5 hours. Outside of this consideration, there appears to be no choice between the two solvents.

Isopropyl and *sec*-butyl alcohols are better solvents for the chlorinated hydrocarbon compounds listed in Table I than is ethyl alcohol. This is an aid to the decomposition of these compounds. Difficulty has been experienced in analyzing 1,2,4,5-tetrachlorobenzene because of its low solubility in ethyl alcohol (*2*), whereas no difficulty of that nature has been experienced with either isopropyl or *sec*-butyl alcohol.

No formation of color has been noticed during the decomposition of the samples in these alcohols, even when "technical" grades have been used. Thus, the inconvenience of specially purifying the solvent is avoided.

tert-Butyl alcohol proved to be an unsatisfactory solvent because of the low solubility of its sodium salt.

Procedure for Analysis

With Isopropyl Alcohol. About 0.15 to 0.3 gram of sample, depending on the halogen content, was weighed in a short glass vial and placed in a 200-ml. Erlenmeyer flask provided with a ground-glass joint, and 25 ml. of isopropyl alcohol and 1.8 to 2.0 grams of sodium, cut into 4 to 5 pieces, were added. The isopropyl alcohol was Merck's, 98 per cent. It was chloride-free, and was used without further purification. The flask was shaken in order to tip the sample vial onto its side, and then connected to a reflux condenser. The alcohol was gently refluxed for 2 to 2.5 hours with occasional shaking. Excess sodium was then decomposed by cautiously adding water, a few drops at a time, through the condenser. Finally, 60 to 80 ml. of water were added, the flask was cooled, 2 to 3 drops of phenolphthalein solution were added, and the contents were neutralized by adding approximately 6 *N* nitric acid drop by drop. To determine chlorides, the solution was then titrated with 0.1 *N* silver nitrate, using 4 to 5 drops of a 1 per cent solution of dichlorofluorescein as an absorption indicator. Eosin was used as the indicator when bromides were determined (*5*, *7*).

With *sec*-Butyl Alcohol. The procedure was essentially the same as for isopropyl alcohol. The *sec*-butyl alcohol was Eastman Kodak "technical", chloride-free, and was used without further purification. Twenty milliliters of alcohol and 1.5 to 1.7 grams of sodium were used. At the end of 2 to 2.5 hours, excess sodium was usually decomposed by adding 15 to 20 ml. of 95 per cent ethyl alcohol through the condenser. Sixty to 80 ml. of water were then added, cautiously at first. Before the titration for chlorides or bromides, 5 to 10 ml. of 95 per cent ethyl alcohol were added, if the solution was not homogeneous owing to the insolubility of part of the *sec*-butyl alcohol. The presence of undissolved *sec*-butyl alcohol causes a coagulation of the silver halide, which interferes with the use of an absorption-indicator. However, this may be an advantage with the Volhard procedure for determining chlorides.

Analysis of *o*-Fluorodiphenyl. Better conditions for decomposing *o*-fluorodiphenyl, m. p. 74–75°, were obtained by using a 20 × 130 mm. test tube provided with a ground-glass joint. In this case, 10 ml. of *sec*-butyl or isopropyl alcohol and 1.5 grams of sodium were used. The *sec*-butyl alcohol was refluxed for 5 to 6 hours. The isopropyl alcohol was refluxed for 4.5 hours, which resulted in the consumption of most of the sodium and the precipitation of considerable quantities of sodium isopropoxide. Fluoride ion was determined as lead chlorofluoride, essentially by the method of Stark (*11*).

Discussion of Analytical Procedure

The use of the secondary alcohols in the decomposition of stable, slightly soluble organic halides results in a greater precision and accuracy than are obtained by using ethyl alcohol in accordance with published procedures.

In the interests of simplicity and convenience, no attempt has been made to proportion the relative amounts of alcohol, sodium, and sample. This has been done according to empirical formulas in the case of ethyl alcohol (*1*, *3*).

The amount of sodium recommended here is in slight excess of that which is consumed in the 2- to 2.5-hour period of reflux used for the decomposition of chlorine and bromine compounds. This amount should thus be adequate for most types of compounds and for variations in sample size. It may be necessary to increase the amount of sodium used by 0.2 to 0.3 gram for periods of reflux longer than 3 to 4 hours, depending on conditions such as rate of reflux, purity of alcohol, and the reaction vessel.

The 2- to 2.5-hour period of reflux has proved adequate to decompose the more refractory type of chlorine compounds. In many cases this time can be reduced as 1,2,4,5-tetrachlorobenzene has been completely decomposed after 1 hour of reflux, using either isopropyl or *sec*-butyl alcohol as the solvent.

Additional analyses are required on different types of fluorine compounds in order to establish more definitely the best conditions for their decomposition. As many fluorine compounds show great stability, longer periods of reflux will probably be necessary to obtain complete decomposition by this method. In this case, *sec*-butyl alcohol would be the better solvent to use because of the higher temperature and the longer reflux period that is possible before the separation of sodium *sec*-butoxide.

Summary

Isopropyl and *sec*-butyl alcohols are very effective solvents for the determination of halogen in nonvolatile compounds by the sodium-alcohol method.

The application of these alcohols to the analysis of stable fluorine compounds has been indicated.

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Determination of Chloride in Bauxite-Supported Anhydrous Aluminum Chloride Catalysts

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NUMEROUS patents describe the use of catalysts made by impregnating bauxite and other granular adsorbents with anhydrous aluminum chloride. The growing large-scale application of this type of catalyst makes necessary a dependable method of analysis for control during manufacture and for assaying shipments and spent material. The purpose of this paper is twofold: to describe satisfactory practical procedures for the determination of the chloride content of the catalyst, and to discuss the experience gained from several thousand analyses.

For a routine determination of the aluminum chloride content of the catalyst the total chloride may be calculated to aluminum chloride. The total chloride is derived not only from anhydrous aluminum chloride, but also from partially hydrated aluminum chloride, probably basic aluminum chloride, small amounts of hydrogen chloride and ferric chloride, and possibly traces of other metallic chlorides produced by the action of aluminum chloride, hydrogen chloride, and water on the carrier during the impregnation. Of all the methods investigated, extraction with water and distillation with concentrated sulfuric acid, followed by the volumetric or gravimetric estimation of chloride in the extract or distillate, gave the most reproducible results after the procedures were carefully standardized. Both methods give low results, since it is impossible to wash the bauxite free of chloride in the extraction procedure, and impractical to ensure complete volatilization of hydrogen chloride by the hot concentrated sulfuric acid because of the coarse mesh size of the sample and the amounts of sample and sulfuric acid it is feasible to employ in the analysis.

Table I. Comparison of Extraction and Distillation Methods

Samples[a]	Aluminum Chloride: Distillation Method 1	Distillation Method 2	Distillation Method Av. (a)	Extraction Method 1	Extraction Method 2	Extraction Method Av. (b)	Difference (a) − (b)
	%	%	%	%	%	%	%
Unimpregnated bauxite	0.00	0.00	...	0.00	0.00	...	..
1	5.67	5.99	5.83	5.77	5.83	5.80	0.03
2	6.28	6.28	6.28	6.20	6.15	6.18	0.10
3	11.06	11.22	11.14	11.54	11.60	11.57	−0.43
4	12.38	12.27	12.33	12.49	12.18	12.34	−0.01
5	12.78	12.70	12.74	12.22	12.57	12.40	0.34
6	14.52	14.46	14.49	13.97	14.24	14.11	0.38
7	14.40	14.67	14.54	14.53	14.63	14.58	−0.04
8	16.79	16.56	16.68	16.48	16.52	16.50	0.18
9	16.63	16.58	16.61	16.56	16.16	16.37	0.24
10	15.70	15.63	15.67	14.74	14.75	14.75	0.92
11	16.12	16.26	16.19	15.86	15.74	15.80	0.39
12	18.25	18.27	18.26	17.83	17.84	17.84	0.42
13	18.10	17.98	18.04	17.48	17.49	17.49	0.55

[a] Samples 1 to 9, inclusive, were 4/10 mesh; samples 10 to 13, inclusive, were 6/14 mesh.

Analytical Procedures

The two recommended methods are given in detail in the following sections. Typical results are shown in Table I. The analyses were made on commercial preparations of Isocel, a catalyst containing 15 to 20 per cent of aluminum chloride on low-iron bauxite, and on samples taken from the impregnators at intermediate stages of manufacture.

Distillation Method. The apparatus illustrated in Figure 1 is constructed from a 500-ml. Kjeldahl flask and a suitable condenser. The 500-ml. absorption flask shown is convenient, although other types of absorption vessels may be used.

Approximately 20 grams of a representative sample are rapidly transferred to a 30-ml. weighing bottle and weighed to the nearest milligram. The contents of the weighing bottle are added to the flask through a wide glass tube, flared at the top, and extending a few centimeters below the bottom of the neck of the flask. The addition tube is withdrawn, the flask is clamped in position, 200 ml. of 18 N sulfuric acid are added rapidly, and the condenser and absorption vessel (containing 200 ml. of water) are quickly fitted into place.

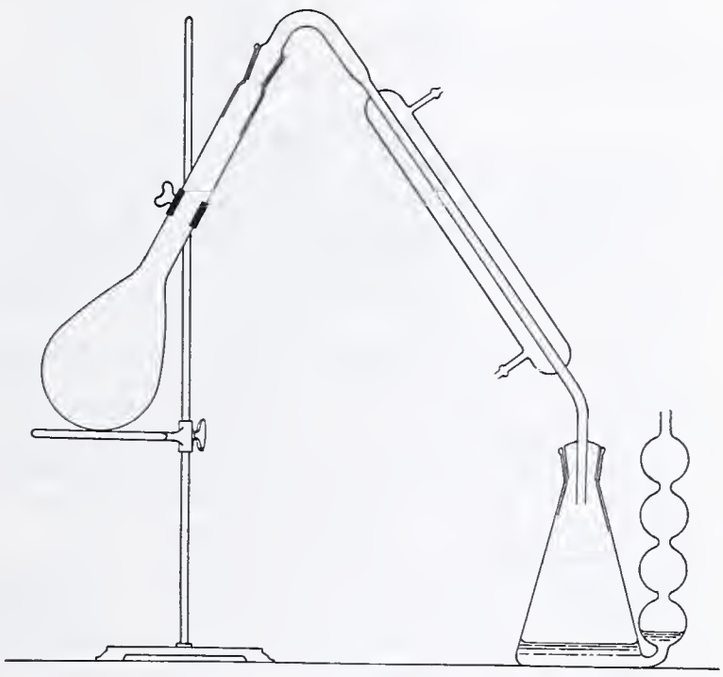

Figure 1. Apparatus for Distillation Method

The mixture is then heated carefully, with occasional shaking, to control foaming. The distillation is continued for 5 minutes following the first appearance (usually after 20 to 25 minutes) of an oily condensate (sulfuric acid) in the upper part of the condenser. During the period of heating approximately 100 ml. of distillate collect in the receiver. The condenser is then removed from the flask and washed down into the absorption vessel with two 25-ml. portions of water. The contents of the absorption vessel are washed into a 1-liter volumetric flask and the volume is adjusted to the mark. A 25-ml. aliquot is transferred to a 250-ml. Erlenmeyer flask, 1.5 ml. of concentrated nitric acid, 25 ml. of 0.1 N silver nitrate, and 1 ml. of saturated ferric alum solution are added, and the mixture is titrated with 0.1 N ammonium thiocyanate according to the directions of Kolthoff (*1*).

Extraction Method. Approximately 50 grams of sample are weighed into a 60-ml. low-form weighing bottle. The weighing bottle is then opened under 300 ml. of cold distilled water in a 800-ml. tall-form beaker (or the sample may be rapidly poured into the water). The bottle and stopper are removed from the mixture, which is then heated to the boiling point with mechanical stirring at a rate which keeps the granules in suspension. The boiling is maintained for exactly 1 minute. The mixture is then allowed to settle for 1 minute and the supernatant liquid (bearing some finely divided bauxite in suspension) is decanted into a 1-liter volumetric flask. Three hundred milliliters of water are added to the residue in the beaker and the

heating, stirring, boiling, and decantation are repeated. The residue in the beaker is washed into a mortar and the lumps are crushed to a coarse powder. The material is then washed back quantitatively into the beaker, the volume adjusted to approximately 200 ml., and the extraction procedure again repeated. The contents of the volumetric flask are refrigerated to room temperature, and made up to 1000 ml. The chloride content is determined as described under the distillation method.

The data in Table I show that the distillation method usually gives higher results than the extraction method, irrespective of the chloride content. The difference between the results obtained by the two methods is greater for the preparations of finer mesh size. The mean variation between duplicate determinations on each of a series of 13 samples was 0.12 for the distillation method and 0.14 for the extraction method.

Table II. Chloride Content in Relation to Size of Sample (Distillation method)

Sample No.	Aluminum Chloride 10-gram sample	20-gram sample	50-gram sample
	%	%	%
21	12.18	11.48	10.12
22	16.03	15.55	14.37

Discussion

Distillation Method. In determining the conditions for the distillation method it was necessary to consider the size of the sample, the amount of acid used for the digestion, the mesh size of the sample, and the means for detecting when the evolution of hydrogen chloride could be considered complete.

It was found that reproducible results could be obtained by heating the mixture for 5 minutes following the first appearance of a few drops of oily distillate (sulfuric acid) in the upper part of the condenser. At this point the supernatant liquid in the flask is chloride-free, but the granules of catalyst remaining undisintegrated contain chloride which can be detected after prolonged soaking in water. The chloride can be entirely expelled from the unground catalyst by using 20 ml. of 18 N sulfuric acid for each gram of sample and continuing the digestion until the bauxite is completely decomposed. This procedure is impractical for a routine analysis, since a 20-gram sample would require 400 ml. of sulfuric acid and several hours' digestion at fuming temperature. It follows that higher results should be obtained by decreasing the size of the sample without changing the amount of sulfuric acid used for the decomposition. The data in Table II show the average values obtained for the chloride content by varying the size of the analytical sample while otherwise following the recommended procedure. Experience indicates, however, that samples smaller than 20 grams should not be used because of excessive variation due to sampling difficulties; lack of reproducibility and seriously low results (due to variation in the amount of chloride retained by the bauxite) are encountered when samples substantially larger than 20 grams are used.

Extraction Method. The amount of chloride found by this method varies with the number of extractions and the state of subdivision of the sample. Higher values are obtained by extracting a finely ground sample, but serious errors are introduced if the sample is ground prior to the first extraction; however, after one or more extractions the granules may be reduced to powder without loss of chloride. There is no detectable loss of hydrogen chloride during the boilings. The volumes of water recommended for the extractions are optimum; larger amounts are unnecessary and inconvenient, while the use of smaller portions results in lower values for the chloride content. The data in Table III show that two extractions followed by crushing and a third extraction give higher values than one to three extractions without crushing, or one extraction with subsequent crushing and one re-extraction. The extracted residues were shown by the distillation analysis to contain residual chloride equivalent to 8 to 9 per cent of the total chloride content—i. e., 1.0 to 1.6 per cent of aluminum chloride for samples containing 13.5 to 19.5 per cent of (total) aluminum chloride (Table III).

Although the distillation method and the extraction method as applied directly to the sample give low results, the total chloride content can be calculated from the sum of the values obtained by the standard extraction of the original sample and the standard distillation procedure applied to the ground residue from the extraction. The following compilation compares the results obtained by the standard methods and the combination procedure. The numbers in parentheses indicate the percentage of chloride detected by the different techniques. While a combination of the two methods will indicate the total chloride content of the catalyst, it is too time-consuming to be recommended for routine plant control procedure:

	Per Cent Aluminum Chloride Sample 14	Sample 15	Sample 16	Sample 17
Standard extraction plus distillation of extracted residue	17.84 (100)	13.52 (100)	16.37 (100)	19.49 (100)
Standard distillation	16.68 (93.5)	12.49 (92.3)	15.67 (96.4)	18.26 (93.5)
Standard extraction	16.58 (92.7)	12.42 (91.9)	14.75 (90.5)	17.84 (91.5)

Soluble Iron Content of Catalyst. The data in Table IV show that 12 to 15 per cent of the iron content of the carrier is converted into soluble form (probably ferrous and ferric chlorides) by the action of the aluminum chloride and hydrogen chloride during impregnation. The iron contributed by the aluminum chloride may be neglected, since the chemical used in the manufacture of these samples contained only 0.1 per cent iron (as Fe_2O_3). The bauxite used as carrier contained not more than 2.5 per cent Fe_2O_3 (volatile-free basis). The samples were processed by the extraction method and the extract and extracted residue were analyzed for iron. The iron values are calculated on the basis of the "as received" catalyst sample.

Sampling. The sample taken in the plant for analysis should be as large and as representative as possible. It is delivered to the laboratory in a tightly-stoppered glass container and subdivided with a 16 to 1 splitter contained in a

Table III. Variation of Chloride Content with Various Extraction Procedures

No.	Treatment: Extractions before crushing	Extractions after crushing	Aluminum Chloride Content: Sample 14[a] Original sample	Sample 14[a] Extracted[b] residue	Sample 15[a] Original sample	Sample 15[a] Extracted[b] residue	Sample 16[a] Original sample	Sample 16[a] Extracted[b] residue	Sample 17[a] Original sample	Sample 17[a] Extracted[b] residue
			%	%	%	%	%	%	%	%
1	2	1	16.58	1.26	12.42	1.10	14.75	1.52	17.84	1.65
2	3	.	16.39	1.09	12.19	0.95	...	..	...	..
3	2	.	16.20	1.35	11.87	1.10	...	..	...	...
4	1	1	16.17	1.48	12.00	1.25	...	..	...	..

[a] Samples 14 and 15 were 4/8 mesh; samples 16 and 17 were 6/14 mesh.
[b] Determined by distillation method.

Table IV. Distribution of Iron in the Extraction Method of Analysis

Sample	% Fe as Fe_2O_3 (a) In extract	(b) In extracted residue	Total Fe as % Fe_2O_3 (a) + (b)	Direct analysis of catalyst
18	0.24	1.74	1.98	1.87
19	0.24	1.66	1.90	1.90
20	0.35	2.05	2.40	2.23

"dry box". The small fraction is transferred to a glass container for analysis. The analytical sample is taken by inserting a test tube (holding 20 or 50 grams under the sampling conditions) into the bottle to the bottom, thereby removing a reasonably representative portion of the material for transfer to the weighing bottle.

Summary

Decomposition of the catalyst with sulfuric acid and removal of the liberated hydrochloric acid by distillation, with subsequent argentiometric estimation of chloride, give reproducible results representing 92 to 96 per cent of the total chloride content.

Reproducible but low results for the chloride content of bauxite-supported aluminum chloride catalysts may be obtained by water-extracting the catalysts by a standardized procedure. The method indicates 90 to 93 per cent of the total chloride content of the sample.

The total chloride content of the catalyst may be determined by a combination of the two methods: standard extraction of the original sample plus standard distillation of the ground residue from the extraction.

Either the extraction or the distillation method is proposed as a satisfactory routine procedure. The combination method may be used when it is essential to know the absolute chloride content.

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Determination of Iron

In the Presence of Chromium and Titanium with the Jones Reductor

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Sulfuric acid solutions of titanous and chromous sulfates, obtained by passage through the Jones reductor, are oxidized by aeration for from 5 to 10 minutes in the presence of a trace of copper sulfate as a catalyst.

Ferrous sulfate is essentially unoxidized and is titrated with permanganate after aeration.

Best results are obtained by using 0.0003 millimole of copper sulfate in about 300 ml. of solution. Larger quantities of copper sulfate lead to slightly low results when both chromium and titanium are present.

THE Jones (*6*) reductor was originally proposed as a rapid and convenient device for the reduction of ferric to ferrous salts, prior to titration with a standardized solution of an oxidizing agent. The solution to be reduced is preferably a sulfuric acid solution, because side reactions that may occur in the presence of hydrochloric acid are thereby avoided in the ensuing titration.

Although the Jones reductor is convenient, several elements other than iron are also reduced by zinc, and various methods have been proposed to eliminate the effect of these interfering elements. Previous studies have dealt mainly with the interference of titanium.

The stability to air-oxidation of ferrous sulfate in sulfuric acid solution was studied by Baskerville and Stevenson (*2*), who showed that practically no oxidation of the ferrous sulfate occurred after 3 hours of aeration. They observed also that the presence of cobalt, chromium, copper, and titanium salts had no effect upon the air-oxidation of the ferrous sulfate. Thornton and Roseman (*8*) studied the preferential oxidation by air of titanous sulfate in the presence of ferrous sulfate. Their results were good, but they suggest that "the procedure is most apt to succeed when the iron is equal to, or preponderates over, the titanium". Gooch and Newton (*4*) used bismuth trioxide for the preferential oxidation of the titanium. Their method requires the removal of the excess of bismuth trioxide before the estimation of iron. Brandt (*3*) used titanium trichloride as the reducing agent for ferric ion, the excess titanium trichloride being destroyed by copper sulfate. In this procedure the cupric ion is reduced to metal by the titanium trichloride and the precipitated copper is filtered off before titrating the iron.

That simple air-oxidation of a titanous solution is not dependable is shown by McNabb and Skolnik (*7*). Their results corroborate the experience of Margaret D. Foster of this laboratory, who found that in a solution containing titanous salt equivalent to 0.04 gram of titanium dioxide less than three fourths of the titanium was reoxidized to the quadrivalent state after 10 hours of aeration; a solution containing 0.09 gram of titanium dioxide was less than nine-tenths converted in the same time. Axt and Leroy (*1*) increased the rate of oxidation by using a perforated plate for supplying the air.

McNabb and Skolnik (*7*) found that the addition of 50 ml. of saturated mercuric chloride solution greatly increased the rate of oxidation of titanous sulfate by aeration.

The method described below is based on the discovery by Zintl and Wattenberg (*10*) that copper in solution catalyzes air-oxidation of titanous ion. This method is applicable for all proportions of iron, titanium, and chromium. Molybdenum and vanadium should be absent.

Table I. Determination of Iron in the Presence of Chromium and Titanium
(3 ml. of 0.0001 M $CuSO_4$ used)

Experiment No.	Fe Taken	TiO_2 Taken	Cr_2O_3 Taken	Aeration	Fe Found	Error
	Gram	*Gram*	*Gram*	*Min.*	*Gram*	*Gram*
1	0.0315	None	None	10	0.0317	+0.0002
2	0.1576	None	None	10	0.1576	None
3	0.0314	0.5000	None	15	0.0316	+0.0002
4	0.0314	0.0030	None	5	0.0313	−0.0001
5	0.0942	0.0030	None	5	0.0940	−0.0002
6	0.2512	0.0030	None	5	0.2511	−0.0001
7	0.2512	0.5000	None	15	0.2513	+0.0001
8	0.0314	None	0.0030	10	0.0316	+0.0002
9	0.0314	None	0.5000	10	0.0314	None
10	0.1256	None	0.0030	10	0.1255	−0.0001
11	0.1256	None	0.5000	10	0.1254	−0.0002
12	0.0630	0.0010	0.0010	5	0.0630	None
13	0.0314	0.0030	0.0030	5	0.0314	None
14	0.1570	0.0030	0.1200	10	0.1566	−0.0004
15	0.0314	0.3000	0.0400	10	0.0313	−0.0001
16	0.0315	0.1400	0.1400	10	0.0312	−0.0003
17	0.0315	0.0400	0.3000	10	0.0313	−0.0002
18	0.1260	0.1400	0.0400	10	0.1259	−0.0001
19	0.1577	0.0400	0.0400	10	0.1573	−0.0004
20	0.0630	0.0400	0.1400	10	0.0628	−0.0002
21	0.1265	0.0400	0.3000	10	0.1265	None
22	0.0316	0.2000	0.0030	10	0.0316	None
23	0.1256	0.0030	0.0030	20	0.1256	None

Apparatus

Jones Reductor, as described and illustrated by Hillebrand and Lundell (*5*). The 20- to 30-mesh zinc is amalgamated with 3 per cent mercury by shaking with a solution of mercuric chloride.

Reagents

Copper Sulfate Catalyst. Solution A, 0.25 gram of cupric sulfate pentahydrate in 500 ml. of water. Solution B, 0.0001 molar cupric sulfate, 5 ml. of solution A in 100 ml. of water. Solution B should be made up fresh occasionally.

Potassium Permanganate Solution, 0.05 N, aged at least a week and filtered through asbestos. Standardized against Bureau of Standards sodium oxalate.

o-Phenanthroline Indicator (*9*), 0.75 gram of *o*-phenanthroline monohydrate and 25 ml. of 0.05 N ferrous sulfate diluted to 50 ml.

Procedure

Pass the iron solution at room temperature (about 200 ml., containing 10 ml. of concentrated sulfuric acid) through the Jones reductor at a rate sufficiently slow to ensure complete reduction (about 100 ml. per minute). Add to the reduced solution 3 ml. of 0.0001 molar copper sulfate and pass through the solution as rapid a stream of air as is possible without loss by splashing. Continue the aeration for 5 minutes after the violet color of titanous sulfate has disappeared. If the violet color is masked by the green of chromium salts, aerate the solution for 10 minutes. If the solution is essentially colorless after reduction aerate for only 5 minutes.

After aeration, titrate the solution of ferrous sulfate in the following manner:

First prepare an indicator solution by adding 0.05 N potassium permanganate, a portion of a drop at a time, to a solution containing 1 to 4 drops of the *o*-phenanthroline indicator in about 10 ml. of 10 per cent sulfuric acid, until the red color just turns to blue. Pour into this indicator solution a portion of the solution to be titrated, and set aside. Titrate the main portion of the solution to the appearance of the permanganate purple color (if much chromium is present the solution becomes gray-green), add the reserved portion, and titrate to the disappearance of the red color of the indicator. Calculate the iron after subtracting a blank determination on the reagents used.

In the absence of much chromium the solutions may be titrated, but with less accuracy, without the *o*-phenanthroline indicator.

Experimental

For the experimental work solutions were made containing known quantities of iron, titanium, and chromium by mixing various volumes of standard solutions. The strength of the iron standard was established by reduction in the Jones reductor and titration with permanganate. Colorimetric tests with hydrogen peroxide showed titanium to be essentially absent in the iron solution. The titanium standard was prepared from reagent grade potassium titanium fluoride (the fluorine being expelled by repeated fuming with sulfuric acid) and the titanium content established gravimetrically. Colorimetric tests with thiocyanate showed no iron in the titanium solution. Chromium was added as potassium dichromate solution made from the reagent grade salt by direct weight. Standardized pipets and burets were employed.

Preliminary studies were made to establish the optimum concentration of copper sulfate to catalyze the oxidation. Large concentrations of copper sulfate, up to 1 mole, caused some oxidation of ferrous sulfate when both titanium and chromium were present, giving a maximum error in ten experiments of −0.0010 gram of iron. However, nine experiments in which only chromium or only titanium was present with the iron showed a maximum error of ±0.0001 gram of iron.

Table I shows the results obtained by adding 3 ml. of 0.0001 molar copper sulfate as catalyst. The results are seen to be accurate in all the experiments made and throughout extreme ranges of composition. Experiments using still less copper sulfate showed somewhat incomplete oxidation of titanous sulfate after 10 minutes' aeration. In the last experiment in Table I the aeration period was increased to 20 minutes and showed no loss in ferrous content through oxidation. In experiments 3 and 7, in which 0.5 gram of titanium dioxide was taken, the violet titanous sulfate color persisted for 9 minutes; consequently the aeration period was extended to 15 minutes.

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Extraction of Ascorbic Acid from Plant Materials

Relative Suitability of Various Acids

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THE stabilization of ascorbic acid during its extraction and determination has long been a problem. Many different extracting acids have been recommended, such as trichloroacetic acid by Birch, Harris, and Ray (*3*), acetic by Bessey and King (*2*), metaphosphoric by Fujita and Iwatake (*6*), oxalic by Watanabe (*14*), sulfosalicylic by Okrent and Wachholder (*13*), metaphosphoric plus trichloroacetic by Musulin and King (*12*), metaphosphoric plus sulfuric by Mack and Tressler (*11*), and various other mixtures. Of these extractants metaphosphoric acid appears to be in most general use at present, and has been found in this laboratory by Loeffler and Ponting (*9*) to be satisfactory for extracting a wide range of plant materials. However, there is little information in the literature on the relative ability of the various acids to stabilize ascorbic acid solutions, and there is some difference of opinion as to which acid is most satisfactory for the purpose.

Watanabe (*14*) first recommended oxalic acid as the best extractant for ascorbic acid, but in a later paper (*15*) he advised the use of a mixture of metaphosphoric and oxalic acids. Lyman, Schultze, and King (*10*) compared metaphosphoric, orthophosphoric, sulfuric, and hydrochloric acids as extractants in the presence of added copper and found metaphosphoric acid the best of this group. Willberg (*16*) compared the stability of ascorbic acid in oxalic, acetic, citric, and tartaric acids and found oxalic acid to be the best of the group. Krishnamurthy and Giri (*8*) compared the stability of ascorbic acid in many carboxylic and hydroxy acids in the presence of acetate buffer and copper, and found the stability greatest in oxalic acid. Metaphosphoric acid was not included in this comparison. Krishnamurthy (*7*) studied further the retardation by oxalic acid of ascorbic acid oxidation (copper, iron, and enzyme-catalyzed), also employing acetate-buffered solutions of pH 5.6. He stated that the degree of retardation produced by oxalic acid is much higher than that produced by either metaphosphoric acid or sodium pyrophosphate at corresponding concentrations, but gave no supporting data.

The experiments herein described were undertaken in order to establish the relative merits of most of the recommended acids and also other common acids as extractants of ascorbic acid, with the possibility of finding a suitable substitute for metaphosphoric acid. Another readily available acid would be desirable because of the present difficulty in obtaining metaphosphoric acid and because of its expense.

Experimental

The method used for the ascorbic acid determinations in making these comparisons was that of Loeffler and Ponting (*9*), in which the ascorbic acid is determined in dilute unbuffered acid by measuring the reduction of the dye 2,6-dichlorophenolindophenol with a photoelectric colorimeter. Since the method employs a pure acid extractant without added buffer, the direct comparison of acids is simple and less interpretation of results is necessary than in the case of buffered extractants.

Too high a concentration of any of the acids causes bleaching of the dye (in the absence of ascorbic acid) and therefore makes the determination difficult and subject to error because of the resulting drift in the galvanometer reading. Since the effectiveness of the various acids in stabilizing ascorbic acid in solution tends to increase with their concentration, it is desirable to use the greatest concentration that does not introduce an appreciable error due to bleaching of the dye. This optimum concentration was determined for each acid as follows:

The rate of drift in galvanometer reading of the standard 1 + 9 mixture of acid and dye was measured (with the Evelyn colorimeter) in the absence of ascorbic acid, using a series of different acid concentrations. That concentration of acid was selected which caused a drift in galvanometer reading of not over $^1/_2$ division per minute (1 division = 1 per cent transmission), since in the determination the readings are made in 15 seconds to the nearest $^1/_4$ division. Therefore, there was no error caused by fading of the dye. The acid concentrations selected are listed in Table I. These concentrations are only approximately correct, since a variation of about 5 per cent is not important. They are for use with pure ascorbic acid solutions and do not apply to the extraction of plant tissue. In the latter case the acid concentrations can be at least doubled without causing bleaching of the dye.

With the above criterion of suitable acid concentration, sulfuric acid can be ruled out at once as unsatisfactory, since it has the peculiar property of causing rapid bleaching of the dye even at concentrations too low to turn the dye red, and far too low to stabilize ascorbic acid.

In determining the stabilizing effect of each acid on ascorbic acid the following procedure was employed:

Approximately 8 mg. of crystalline ascorbic acid were weighed and dissolved in acid of suitable concentration as determined above, to give 250 ml. of solution containing about 3.2 mg. of ascorbic acid per 100 ml. Two 50-ml. portions were set aside in stoppered 250-ml. flasks at room temperature (about 23° C.). Since it is well known that copper catalyzes the oxidation of ascorbic acid, copper sulfate pentahydrate was added to another 100-ml. portion to give a cupric-ion concentration of 10^{-4} *M*. This solution also was divided into 50-ml. portions and set aside. The remaining 50-ml. portion of the original solution was used to determine the pH and initial concentration of ascorbic acid. After 24 hours the residual ascorbic acid was measured in the

Table I. Loss of Ascorbic Acid from Pure Solutions in Various Acids

Acid	pH	Initial Ascorbic Acid	No Copper Added: Ascorbic acid after 24 hours	No Copper Added: Av. loss	10^{-4} *M* Copper Added: Ascorbic acid after 24 hours	10^{-4} *M* Copper Added: Av. loss
		Mg. %	*Mg.* %	%	*Mg.* %	%
0.5% metaphosphoric	2.10	3.26	3.16 3.18	2.8	2.79 2.79	14.4
0.2% oxalic	1.86	3.24	3.14 3.16	2.8	2.92 2.92	9.9
0.07% perchloric	1.84	3.24	2.76 2.78	14.5	0.17 0.10	95.7
1% citric	2.17	3.32	2.58 2.46	24.1	0.08 0.08	97.6
0.5% tartaric	2.21	3.26	2.42 2.46	24.5	0.17 0.17	94.8
0.07% nitric	1.88	3.23	2.50 2.50	25.7	0.18 0.18	94.4
5% acetic	2.30	3.28	2.16 2.34	31.4	0.03 0.00	99.5
0.2% maleic	1.97	3.32	2.05 2.22	35.6	0.00 0.04	99.4
0.5% sulfosalicylic	1.64	3.26	1.79 1.77	45.4	0.71 0.71	78.3
0.2% trichloroacetic	2.07	3.22	1.71 1.72	46.6	0.08 0.08	97.5
1% lactic	2.01	3.26	1.39 1.33	58.3	0.65 0.55	81.6
0.07% hydrochloric	1.68	3.24	1.34 1.17	61.1	0.02 0.02	99.4
0.2% orthophosphoric	1.87	3.26	0.45 0.45	86.2	0.02 0.00	99.7
Water	4.27	3.29	0	100	0	100

solutions which had been set aside. The solutions without added copper were tested for copper with sodium diethyldithiocarbamate and found to give negative results in all cases. The solutions were made up in water twice distilled from glass and giving no test for copper even when concentrated 1000 to 1.

The results of these experiments are listed in Table I. The only acids showing low losses with pure solutions of ascorbic acid are seen to be metaphosphoric and oxalic. These acids therefore were used to extract the ascorbic acid from a series of vegetables and fruits in order to check the consistency with which the acids exerted a stabilizing effect in different plant extracts and in the presence of ascorbic acid oxidase.

Twenty-five to 50-gram samples of plant tissue, depending on its ascorbic acid content, were blended in a Waring Blendor for 5 minutes in 450 ml. of extracting acid of concentration shown in Table II and suction-filtered. About 125 ml. of filtrate were obtained and two 50-ml. portions were set aside in 250-ml. glass-stoppered flasks. The pH and original ascorbic acid were determined using the remaining 25 ml. of filtrate. In the case of cabbage, broccoli, and strawberries more filtrate was obtained and two portions also were set aside with added cupric ion at a concentration of 10^{-4} M. After 24 hours the ascorbic acid was measured in the flasks which had been set aside. The results of these experiments are shown in Table II.

Table II. Loss of Ascorbic Acid from Extracts of Plant Materials in Oxalic and Metaphosphoric Acids

Plant Material	Extracting Acid	pH of Filtrate	Ascorbic Acid Initial	Ascorbic Acid After 24 hours	Av. Loss
			Mg./100 ml.[a]		%
Fresh cabbage	0.4% oxalic	1.79	3.56	3.14, 3.16	11.5
	0.4% oxalic + Cu	1.79	3.56	3.18, 3.20	10.4
	1% metaphosphoric	1.91	3.33	3.20, 3.22	3.6
	1% metaphosphoric + Cu	1.91	3.33	2.95, 2.95	11.4
Frozen broccoli	0.4% oxalic	1.87	3.52	3.12, 3.14	11.1
	0.4% oxalic + Cu	1.87	3.52	3.18, 3.16	9.9
	1% metaphosphoric	1.97	3.37	3.08, 3.06	8.9
	1% metaphosphoric + Cu	1.97	3.37	2.88, 2.96	13.4
Frozen lima beans	0.4% oxalic	2.37	1.67	1.47, 1.42	13.5
	1% metaphosphoric	2.41	1.76	1.39, 1.39	21.0
Frozen peas	0.4% oxalic	2.02	1.65	1.08, 1.09	34.2
	1% metaphosphoric	2.17	1.52	1.11, 1.17	25.0
Frozen strawberries	0.4% oxalic	1.68	3.55	3.04, 3.03	14.5
	0.4% oxalic + Cu	1.68	3.55	2.97, 2.97	16.3
	1% metaphosphoric	1.88	3.12	2.90, 2.90	7.1
	1% metaphosphoric + Cu	1.88	3.12	2.55, 2.55	18.3

[a] Mg. of ascorbic acid per 100 grams of plant tissue = mg. per 100 ml. of filtrate × 18.9 for cabbage, broccoli, and strawberries; and mg. per 100 ml. of filtrate × 9.8 for lima beans and peas.

Discussion

As can be seen from the duplicate values listed in Table I, the reproducibility of results is satisfactory under the conditions employed and the values are comparable. With plant extracts (Table II) the losses in each acid are reproducible and the initial and final values are comparable, but the initial ascorbic acid values in one acid are not to be compared with those in the other acid because no effort was made to obtain completely uniform samples of plant tissue. With uniform samples the ascorbic acid values are the same with either metaphosphoric or oxalic acid. In either acid there is no loss of ascorbic acid during blending for the usual 5 minutes; in fact, at the start of this work a 10-minute blending period was used to promote oxidation, but this method was abandoned because the losses in metaphosphoric and oxalic acids were within the experimental error, even though the temperature reached 45° C.

It is obvious that the stability of ascorbic acid solutions is not merely a function of pH but depends also on the nature of the acid (Table I). Thus metaphosphoric acid with a pH of 2.1 shows a 2.8 per cent loss, in the absence of copper, and orthophosphoric acid with a pH of 1.87 shows a loss of 86.2 per cent under similar conditions.

Oxidation of pure ascorbic acid in water solution was complete at pH 4.27, although the water contained a copper concentration of less than 5×10^{-9} M. One hundred milligrams of the ascorbic acid in 10 ml. of water gave a negative test for copper; this was about 300 times the concentration used in the experiments.

From the results listed in Table I, it is evident that the only acids of the group listed which are satisfactory for the extraction of ascorbic acid are oxalic and metaphosphoric. Oxalic acid seems to be the more effective of the two in inhibiting oxidation in the presence of copper, whether ascorbic acid oxidase is present or not (Tables I and II). With some vegetable extracts in oxalic acid (Table II) the loss of ascorbic acid in the presence of added copper was less than in its absence. No explanation is offered for this, but it appears to be a rather consistent phenomenon. In the absence of copper the losses of ascorbic acid in plant extracts were generally a little less in metaphosphoric than in oxalic acid.

In a series of tests with plant extracts in 0.5 per cent oxalic acid and 1 per cent metaphosphoric acid, the losses were generally a little less in oxalic than in metaphosphoric acid. The concentration of either acid can be markedly increased in the presence of plant extracts without causing bleaching of the dye, but even in vegetables having a high pH it has not been found necessary to use a concentration greater than 0.5 per cent for oxalic acid or 2 per cent for metaphosphoric acid to prevent oxidation of ascorbic acid during blending. Ordinarily a concentration of 0.3 per cent for oxalic or 1 per cent for metaphosphoric acid is satisfactory, used in a ratio of 7 volumes of acid to 1 of plant material, or higher. This ratio has been found necessary to obtain extraction of the ascorbic acid to equilibrium between the solid and liquid phases (Loeffler and Ponting, *9*).

Both oxalic and metaphosphoric acids prevent enzymic oxidation of ascorbic acid, as shown by an even lower loss in fresh cabbage extract, which contains a relatively large amount of ascorbic acid oxidase, than in extracts of peas or strawberries, which contain practically none. (The frozen vegetables listed in Table II were all blanched prior to freezing.) This is to be expected, since Ebihara (*4*) found no activity of ascorbic acid oxidase below pH 4 and Engelhardt and Bukin (*5*) found none below a pH of about 3.5.

As Lyman, Schultze, and King (*10*) have shown, metaphosphoric acid buffers have no value in stabilizing ascorbic acid above a pH of about 3.3. This is in marked contrast to the stability at a high pH in buffers containing oxalic acid, which has been pointed out by Krishnamurthy (*7*). In confirmation of Krishnamurthy, the author has found almost the same rate of oxidation in a 0.01 M ammonium oxalate buffer at pH 5.6 as in the acid itself at pH 1.86—namely, about 3 per cent in 24 hours under the conditions described above—and for the measurement of ascorbic acid oxidase, a 0.01 M oxalate buffer of pH 6.0 is employed, in which no measurable nonenzymatic oxidation of ascorbic acid occurs in 20 minutes, even though considerable copper (up to 2 or 3 p. p. m.) is present.

There is often a difference in turbidity of oxalic acid and metaphosphoric acid extracts of fruit or vegetable tissue. With some materials oxalic acid gives the clearer filtrate and with some the metaphosphoric acid extract is clearer. In either case the turbidities do not interfere in the colorimetric method used.

Because of the instability of metaphosphoric acid, pointed out by Bessey (*1*), solutions of this acid must be made fresh every few days. Oxalic acid solutions, on the other hand, can be made in large quantities and stored indefinitely.

Summary and Conclusion

Of the 13 acids compared as to their stabilizing effect on ascorbic acid solutions under conditions favorable to oxidation, only metaphosphoric and oxalic acids appeared suitable, these two acids being far superior to any of the others and about equally satisfactory. It is concluded that oxalic acid may be safely substituted for metaphosphoric acid in the determination of ascorbic acid, thus providing a more stable, more easily obtainable, and less expensive extractant.

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Apparatus for Purification of Hydrocarbons by Recrystallization

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During the course of an investigation into the properties of normal paraffin straight-chain hydrocarbons, it was necessary to synthesize various homologs and to obtain them in the highest possible state of purity. Purification was accomplished principally by repeated recrystallization from glacial acetic acid, since the higher paraffin homologs are slightly soluble in this acid at boiling temperatures and quite insoluble in the acid at room temperatures. A description of the apparatus used may be of particular interest to those wishing to prepare pure hydrocarbons.

In practice, the impure hydrocarbon was dissolved by heating to the boiling point 1 to 3 grams of the hydrocarbon per liter of acid. Upon cooling, the hydrocarbon crystallized out in a mass of small, white needles. This procedure was repeated until the sample gave a constant melting point. In some cases it was found necessary to repeat the crystallizations as many as twenty times, the final melting point being approached asymptotically.

In order to facilitate the operation, which is rather cumbersome and tedious when carried out by the usual method, and to reduce the possibility of contamination from outside sources, the apparatus shown in Figure 1 was devised.

Two 5-liter flasks were connected across a condenser, as shown. The hydrocarbon to be purified was dissolved in pure glacial acetic acid and poured into flask 2 through the condenser, vacuum being applied to the system through *A* and *C*. When this method was not practical, as in the case of hydrocarbons with high melting points, flask 2 was filled with the acid and the solid hydrocarbon was dropped down through the condenser. Flask 2 was then heated and the mixture allowed to reflux for several hours. In this way it was possible to purify by recrystallization up to 15 grams of the hydrocarbon at one time.

When the acid was allowed to cool to room temperature, the hydrocarbon crystallized out and collected as a diffuse, white, cloudlike layer on top of the acid.

Section *F* consisted of 10 cm. of 2.5-cm. bore Pyrex, joined to 2-mm. bore tubing. This tubing was connected through stopcock *D* to flask 1. The expanded section, *F*, was filled with acid-washed glass wool and so packed as to provide a filter for the hydrocarbon crystals and whatever solid or insoluble impurity might be included with the sample being purified. With stopcocks *C* and *A* closed, suction was applied through *B*, transmitted through stopcock *D*, through *E* and *F* to the bottom of flask 2. When all the mother liquor had been drawn into flask 1 from

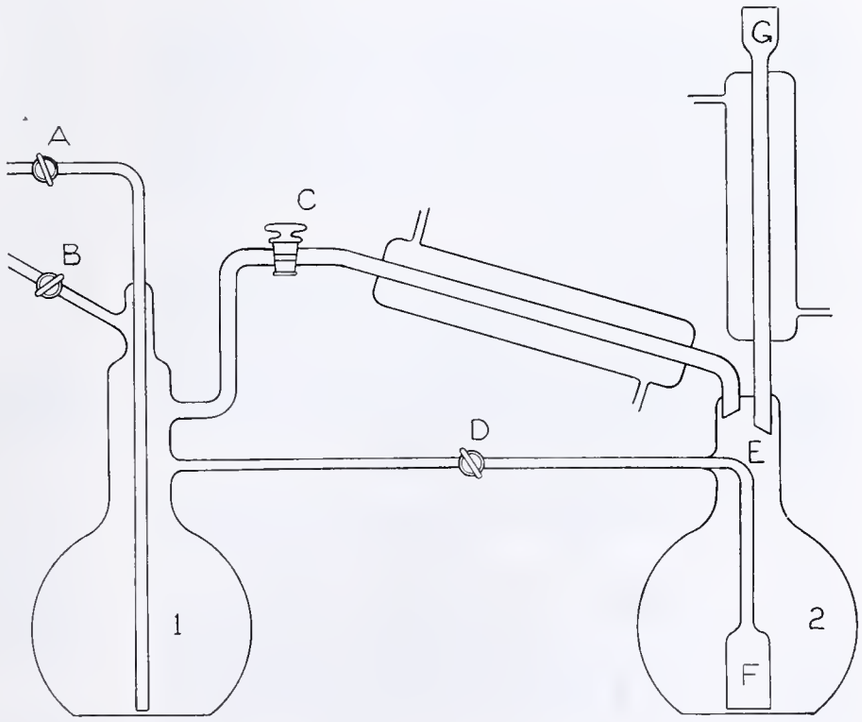

Figure 1. Diagram of Apparatus

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flask 2, stopcocks *B* and *D* were closed, *C* was opened, and heat was applied to flask 1. Acid was distilled into flask 2 until 2.5 or 5 cm. (1 or 2 inches) of impure acid residue remained in the bottom of flask 1.

With successive cycles of operation, the hydrocarbon in flask 2 became purer, and the acid in flask 1 became contaminated with increasing amounts of soluble impurities. At regular intervals, samples of hydrocarbon were withdrawn from flask 2. This was accomplished as follows: When all but 50 to 100 ml. of acid had been drawn into flask 1, a long glass tube was inserted into flask 2 through the mouth of the condenser at *G*, and through this tube several milliliters of acid and crystals were drawn off. The crystals were filtered, washed thoroughly with distilled water, and dried, and a melting point determination was made.

When the hydrocarbon was considered sufficiently pure, as indicated by constant melting point, stopcocks *C* and *D* were closed, pressure was applied through *B* and the impure acid was discharged through *A*. Flask 1 was thoroughly cleaned by repeated washing with hot acetic acid. The cold acid, which by this time contained negligible soluble impurity, was drawn from flask 2 into flask 1, and, as before, blown out through *A*. Flask 2 was then refilled with fresh acetic acid, heated to boiling, and the entire contents of the flask were drawn over into flask 1. From there they were drawn or blown directly into a receiving flask. Alternatively, the purified hydrocarbon could have been drawn directly from flask 2.

All passages were cleared of hydrocarbon by drawing hot acid washings down through *G*, up through *F*, *E*, and *D*, and out at *A*. In order to decrease the possibility of contamination from the stopcock grease, several liters of hot acetic acid were drawn through all stopcocks before the hydrocarbon was introduced into the system.

The apparatus was used only for the purification of hydrocarbons, but it is possible that the same apparatus, or a modification in which the glass wool, *F*, is replaced by a selected glass filter, could be utilized for a variety of laboratory crystallizations. It is particularly convenient where the amount of solvent is large and a number of recrystallizations are necessary.

Filtration Cylinder

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IN QUANTITATIVE analysis there are times when the precipitate must be filtered and washed and the filtrate brought to a required volume. At other times, the precipitate collected on the filter may require resolution, and the solution must be made up to a known volume. This is especially true in colorimetric work (*1*, *2*, *3*). The use of an ordinary suction flask for either of these operations requires rinsing from the suction flask into a volumetric flask, with the possibility that thorough rinsing will give a volume larger than desired.

A review of the literature showed that this problem has been evident to many. The apparatus most nearly approaching the authors' needs was one described by Yagoda (*4*), but this was designed for microanalysis, and no commercial source of the apparatus was indicated.

A piece of apparatus most suitable to the authors' needs was made, and this standard volume filtration cylinder may now be obtained from Emil Greiner, 161 Sixth Ave., New York, N. Y. The cylinder was designed to accommodate a 100-ml. volumetric flask, or a 120-ml. (4-ounce) sample bottle. Figure 1 shows the apparatus in this 100-ml. form. This size could be made to meet almost any need, or a 200-ml. volumetric flask could be shaped to fit the present cylinder. The letters in Figure 1 refer to the following parts:

1. A No. 3 Gooch crucible.
2. An ordinary Gooch crucible adapter for a suction flask. The tip of the glass tube must be drawn out to give a fine stream. This prevents the stream from blocking the air being displaced from the volumetric flask, causing bubbling and loss of sample.
3. A special heavy rubber stopper, with a hole in the center to hold the adapter (2). The shoulder of the stopper rests on the top of the cylinder, making an air-tight seal when suction is applied. This construction prevents the cylinder from being split by the wedge effect of a tapered stopper.
4. Heavy glass cylinder, 22.5 cm. (9 inches) tall and 7.5 cm. (3 inches) in inside diameter.
5. A three-way stopcock permitting suction to be applied or air introduced into the cylinder without disconnecting the suction line.

In using the apparatus, the rubber stopper and adapter are removed from the cylinder. The 100-ml. volumetric flask is placed in the cylinder, the rubber stopper replaced, and the adapter put into position, so that its stem is in the neck of the flask. A prepared Gooch crucible is placed on the adapter, the stopcock is turned to connect the suction line to the cylinder, then the filtration is made, and the precipitate is washed. When the volume of the filtrate and washings is nearly 100 ml., the suction is turned off, the flask removed, and the volume made exactly 100 ml. For work where the volume is not important, a 20-ml. (4-ounce) sample bottle may be used to receive the filtrate.

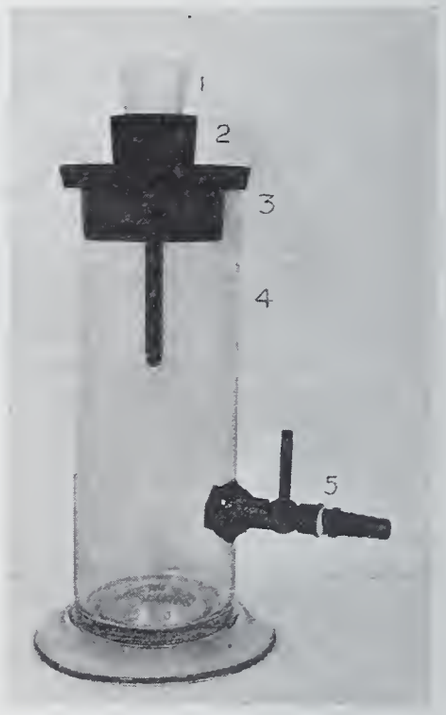

FIGURE 1

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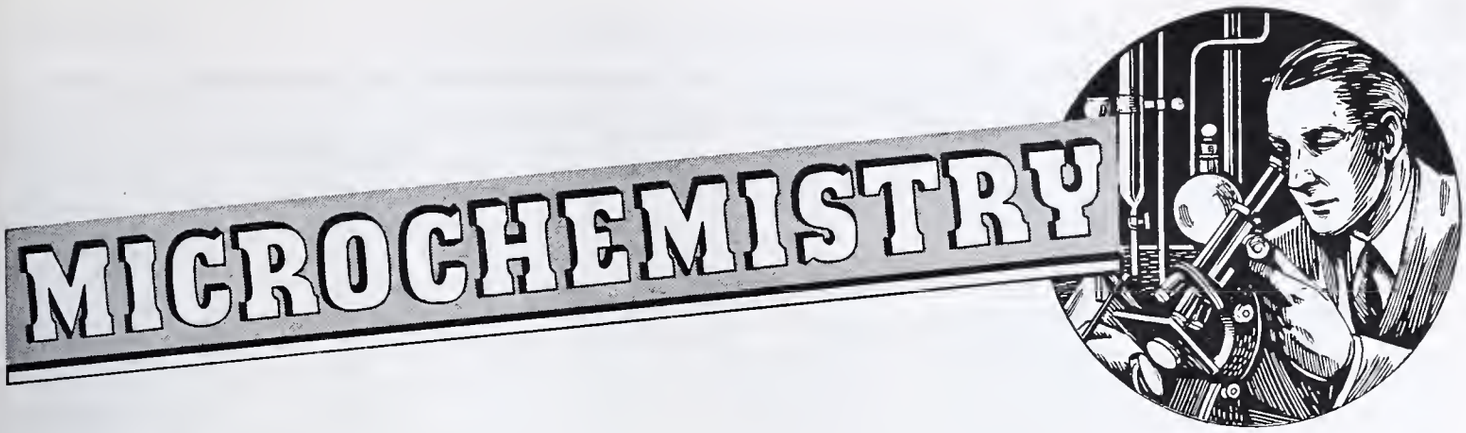

Semimicroanalysis of Saline Soil Solutions

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A system of photometric and volumetric analytical semimicromethods for ions that contribute to soil salinity is described. These methods involve a considerable reduction in the quantity of soil solution required, which is an important consideration in the extraction of such solutions. In addition, they involve a saving of reagents and time. The precision and accuracy of the methods are considered adequate for most soil analyses.

THE detailed analysis of soil solutions is rendered difficult by the large volume of sample required by the standard analytical methods. The method of extraction, size of apparatus, size of soil sample, necessity for repetition of the extraction, and length of time required for extraction have been influenced by the necessity of securing sufficient solution for complete analysis. (In this paper, the term "soil solution" refers to the aqueous solution occurring in the soil at field moisture; the term "soil extract" refers to the solution obtained from a soil that has been mixed with an artificially high quantity of water—e. g., at soil-water weight ratios of 1 to 2 and 1 to 5.) In a discussion of these factors, Eaton and Sokoloff (*10*) pointed out that "a material reduction in the quantity of solution required in the laboratory would minimize some of the difficulties". Anderson, Keyes, and Cromer (*4*) recently mentioned the necessity of altering analytical conditions in the direction of microchemistry.

The staff of this laboratory has been engaged in the examination and development of methods for the extraction of soil solutions, particularly of saline and irrigated soils. The pressure-membrane method, described by Richards (*28*), is an effective means of obtaining solutions from soils covering wide ranges of moisture content, texture, structure, and salt content. This method appears to be especially well adapted to soils at low moisture contents—e. g., near the wilting range (*27*). The advantages of the pressure-membrane method would be largely lost if it were necessary to apply the conventional analytical methods to the limited volumes of solution obtainable from comparatively dry soils.

Consequently, the development of the pressure-membrane method has emphasized the need for semimicroanalytical methods applicable to small samples of soil solution. In addition to the small amount of sample required, the methods outlined here involve a saving of time and reagent, a consideration which might be of even greater importance to some analysts. There is an expanding interest in the application of microanalytical methods to problems of agricultural chemistry. Peech (*24*) recently published a scheme for the microdetermination of exchangeable bases in soils. Wall (*36*) has developed a set of microprocedures for the determination of some inorganic constituents of plant ash.

This article presents photometric and volumetric methods for the semimicrodetermination of calcium, magnesium, sodium, potassium, ammonium, carbonate, bicarbonate, chloride, sulfate, and nitrate ions. These methods generally represent adaptations of other methods previously published for the analysis of soils, waters, plants, and clinical specimens. The methods necessarily vary as to convenience and accuracy. The aim has been to develop simple procedures whose precision would not be seriously less than that of corresponding macromethods.

The methods described apply primarily to saline alkaline soils in which salts of alkali and alkaline earth metals predominate. For use under other conditions, where additional interfering substances might occur, appropriate modifications might be necessary. If the soil solution is not analyzed immediately, the concentration of some ions may be appreciably altered by the activity of microorganisms. Treatments to minimize the direct effect of such processes on the nutrient ions and indirect effects on other ions are usually not reliable. In addition, calcium carbonate and calcium sulfate precipitate from some solutions after extraction. For these reasons, soil solutions should be analyzed as soon as possible.

The centrifuge procedures involve the use of an 8-place centrifuge head rotating at 3000 r. p. m. in a No. 2 International centrifuge. Heavy-duty 12-ml. conical centrifuge tubes are necessary at this high speed in place of the ordinary 15-ml. tubes. A 16-place head rotating at 2000 r. p. m. also provides acceptable results, and may even be more practical for a large number of samples. An angle head rotating at 3000 r. p. m. was tested, but in general the results were inferior with respect to precipitate compaction and over-all accuracy.

A photoelectric photometer is very satisfactory for the colorimetric measurements because of its speed and relative precision. An Aminco Type F double photocell photometer (manufactured by the American Instrument Company, Silver Spring, Md.) was used in this work. It has a permanent mounting of six pairs of matched color filters and permits the use of both optical cells and photometer test tubes. A constant-voltage transformer in the 115-volt power line eliminates fluctuations in light transmission due to voltage variations. Other color-measuring instruments,

Table I. Description of Soils and Their Extracts

Accession No.	Soil Type	Location	pH	C, %	Extract Characteristics: C, %	pH	Electrical conductivity, $K \times 10^5$ @ 25° C.	Soil: water ratio	Color
56[a]	Imperial clay	Meloland, Calif.	7.8	0.34	0.00094	7.8	125	1:2	Very pale yellow
57	Imperial clay	Imperial, Calif.	7.2	0.33	...	7.0	803	1:5	Colorless
62	Oasis clay loam	Delta, Utah	7.7	1.07	...	7.6	998	1:5	Yellow
63[a]	Oasis clay subsoil	Delta, Utah	8.0	0.58	...	8.1	557	1:5	Yellow
68[a]	Vale silty clay loam	Vale, Ore.	10.0	0.27	0.0032[b]	10.2	555	1:5	Brown
79	Cajon silty clay loam	Glendale, Ariz.	7.7	1.10	0.0044	7.3	75	1:2	Yellow
84	Gila adobe clay	Las Cruces, N. Mex.	8.0	0.92	0.0034	8.1	85	1:2	Pale yellow
85	Regan clay loam	Roswell, N. Mex.	7.8	0.93	0.0032	7.6	353	1:2	Pale yellow
86	Fort Collins loam	Laramie, Wyo.	8.0	0.99	0.0053	7.6	544	1:2	Pale yellow
248[a]	Indio very fine sandy loam	Coachella, Calif.	9.1	0.49	0.0047	9.0	?2	1:2	Reddish brown
314	Merced clay loam	Buttonwillow, Calif.	7.8	2.34	0.0047[b]	7.6	625	1:5	Dark yellow

[a] Soils used only for organic matter investigation.
[b] Values corrected for medium chloride contents and probably less accurate than those for other soils.

such as spectrophotometers, gradation photometers, neutral wedge photometers, and visual color comparators, can also be used. For accurate photometric work it is usually hazardous to rely on permanent photometer calibration curves, because of the variable conditions that affect colorimetric procedures. In this laboratory it is a matter of routine to take a series of standards through the analytical procedure each time a group of samples is analyzed.

The calibration of microburets and small pipets is recommended. A 2-liter beaker covered by a 20-hole perforated brass plate, which holds the centrifuge tubes vertical, makes an adequate water bath.

Ionic concentrations are calculated in terms of milliequivalents per liter (m. e./l.). Attention is called to the increasing use among water chemists of the term "equivalent per million", e. p. m. (*3*). This unit of concentration is numerically the same as milliequivalents per liter if the specific gravity of the solution is unity.

In addition to the determination of ionic concentrations, the analysis of soil solutions usually includes the pH value and the electrical conductivity as a measure of the total electrolyte concentration. For conductivity measurements on small samples, a micromodification of the common pipet type of conductivity cell, which holds approximately 5 ml., is very convenient. For pH measurements a Beckman "one-drop" glass electrode (manufactured by National Technical Laboratories, South Pasadena, Calif.) is satisfactory. Capillary glass microelectrodes, which require even less sample, are also available.

Description of Soils and Extracts

The systematic investigations of organic matter and precision and accuracy reported here were made on extracts of eleven soil samples of different soil types from various localities. Pertinent characteristics of these soils and extracts are presented in Table I. Two of these soils, 68 and 248, are "black alkali" soils.

The organic carbon contents of the soils and extracts were determined by the chromic acid oxidation method of Schollenberger (*30*), involving the modified phosphoric acid reagent of Purvis and Higson (*26*). The experimental values were multiplied by the factor 1.15, according to Allison (*1*), which corrects for incomplete oxidation of the organic matter. The carbon contents of the extracts were determined on the evaporation residue of 25-ml. aliquots. Chloride reduces chromic acid, and appropriate corrections are included in the eight extract values reported. The soil carbon contents of the more saline soils also include corrections for chloride, which are very slight compared to those for the corresponding extracts.

The pH values were determined by a glass electrode assembly. The soil pH measurements were made on saturated soil pastes.

Removal of Organic Matter

The possible interference of organic matter in the analysis of soil solutions often raises questions concerning the necessity for its removal. It may interfere in such ways as color masking, reducing action, mechanical contamination of precipitates, and in other direct and indirect ways. The magnitude of these effects is usually unknown. In some systems of analysis, all samples are treated to remove organic matter, regardless of the amount and composition. In other cases the solutions are analyzed without prior separation of the organic fraction.

As the time involved in the preliminary removal of organic matter represents an appreciable fraction of the total time required for analysis, information as to the feasibility of omitting this operation is important, especially in the routine analysis of a large number of soil samples. It is also possible that some or all of the methods for removing organic matter may actually introduce errors into the analytical results. These considerations may affect both macro- and microanalytical methods.

The eleven water extracts of Table I were subjected to four different treatments: ignition, oxidation by hydrogen peroxide, oxidation by bromine, and adsorption by carbon. Other possible treatments were not investigated systematically because they would definitely introduce various kinds of interference. The treated and untreated samples were analyzed for the ions mentioned except nitrate and ammonium. Sodium was determined by a gravimetric uranyl zinc acetate procedure [*39*, Sect. 70 (b), p. 42] instead of the colorimetric method.

Bromine removed the color from all samples, but the analytical values agreed with those of the untreated solutions. Consequently, there would be no advantage in the use of this oxidant in the scheme of analysis described here.

Carbon not only adsorbed the colored constituents but significantly reduced the concentrations of most of the ions, especially calcium and magnesium, and lowered the bicarbonate-carbonate value of every sample. However, it did not affect the chloride values, which indicates that carbon treatment may be useful in removing color that interferes with the chloride titration.

Hydrogen peroxide treatment at a temperature not exceeding 100° C. caused a general decrease of ions in most samples, particularly of sodium and chloride. The loss of chloride is probably a result of oxidation to chlorine. The cause of the losses of the other ions remains somewhat obscure. These results, coupled with the resistance of some organic matter to oxidation by peroxide and the possibility of the catalytic decomposition of peroxide by soil constituents, make this treatment unsatisfactory.

The ignition treatments were made in porcelain casseroles

at 600° C. The more resistant organic constituents did not decompose completely over extended periods at lower temperatures. This has been observed also on base-exchange residue ignitions. The results of ignition were variable. Sodium and chloride were lost from every sample, to about the same extent. Decreases in magnesium and sulfate occurred in several samples. Calcium showed no significant change except an increase in one "black alkali" sample, 68. Potassium was extremely high in ten ignited samples, upwards to 500 per cent of the correct value. Fresh samples of four solutions that were extremely erratic in this respect were ignited in platinum dishes, and these yielded the correct values for potassium. The excess potassium evidently originated in the material of the casseroles; the results indicate an exchange of sodium for potassium.

The two black alkali samples were also treated with nitric acid and boiled, to precipitate the colored humates. Analysis of the filtrates showed no appreciable deviation from the untreated samples.

In this investigation, carbonate determinations were made only on the untreated and the carbon-treated samples, as the other treatments precluded this determination. Chlorides could not be determined on the bromine-treated sample.

The results of this investigation suggest the following recommendations and possible conclusions. For titration of carbonate species in dark solutions, a potentiometric titration can be substituted for the indicator procedure. Purified decolorizing carbon can safely be used to treat dark solutions prior to the chloride titration. Carbon can evidently be used to remove the color of solutions prior to the determination of sodium and potassium. Ignition may cause appreciable loss of many ions common to saline soils, especially sodium and chloride. Ignitions should not be made in porcelain ware. Oxidation of organic matter by bromine and hydrogen peroxide accomplishes no apparent beneficial results. With especial regard to the inorganic composition of black alkali solutions, the inclusions of ions such as calcium and magnesium that may be combined with the humates may not always be desirable.

Precision and Accuracy

To demonstrate the possible ranges of precision and accuracy that can be expected from the various methods, water extracts of seven soils of Table I were systematically analyzed in duplicate by the semimicromethods and by the corresponding macro- or standard methods in use at this laboratory. The results for each particular ion are presented in the section devoted to the d scussion of that method.

Organic matter was not removed from these extracts prior to their analysis by either the macro- or microprocedures. As the extracts vary considerably in composition, some reported determinations may involve quantities of ions that do not represent favorable conditions for the evaluation of the accuracy of a method. This applies also to the macromethods.

In addition to the comparisons reported here, many other similar studies have been made on soil solutions and soil extracts, waters, plant nutrient culture solutions, and plant ash extracts. These studies have yielded results as satisfactory as those presented in this paper.

In the succeeding tables, several symbols and terms are used that possibly require brief explanations. The letters A and B indicate duplicate determinations. The mean is the average of the duplicate values, reported to the same decimal point. The per cent deviation represents the average deviation of the duplicates from the mean divided by the mean value, and times 100; this figure is an index of precision or reproducibility. The average per cent deviation is the arithmetical average of the per cent deviation values for the entire group of extracts; comparison of the two values obtained for two methods provides a measure of the relative precision of the two methods. The per cent error represents the algebraic percentile deviation of the mean semimicro value from the mean macro value; this calculation assumes that the macromethod usually provides the more correct result. The average per cent error is the arithmetical average of the percentile errors for the entire group of semimicrodeterminations; this figure provides a general index of the over-all accuracy of the method.

VOLUMETRIC DETERMINATION OF CALCIUM

Calcium is determined by a method involving precipitation as the oxalate, centrifugal washing, and direct titration in perchloric acid solution with ammonium hexanitrato cerate, with nitro-ferroin as indicator. (The ammonium hexanitrato cerate and nitro-ferroin can be obtained from the G. Frederick Smith Chemical Company, Columbus, Ohio.) The precipitation and washing technique represents a combination of modified Clark and Collip (*9*) and Blasdale (*8*) procedures, while the titration technique follows the procedure of Smith and Getz (*32*). The use of cerate and nitro-ferroin permits a direct titration at room temperature with a very sharp endpoint change from red to pale blue, and a low blank correction.

Reagents. (Keep reagents B, C, D, and E in Pyrex bottles.)

A. Methyl orange, 0.01 per cent in water.

B. 1 to 1 hydrochloric acid.

C. 1 *N* oxalic acid.

D. 1 to 1 ammonium hydroxide.

E. 1 to 50 ammonium hydroxide.

F. 4 *N* perchloric acid. Dilute 340 ml. of 70 per cent perchloric acid or 430 ml. of 60 per cent perchloric acid to 1 liter.

G. 0.01 *N* ammonium hexanitrato cerate in 1 *N* perchloric acid. Dissolve 5.76 grams of "standard or reference purity" ammonium hexanitrato cerate in 250 ml. of 4 *N* perchloric acid and dilute to 1 liter. The reagent should be standardized in the following manner: Pipet 5 or 10 ml. of fresh standard 0.01 *N* sodium oxalate into a small beaker containing 5 ml. of 4 *N* perchloric acid, add 0.2 ml. of nitro-ferroin indicator and titrate with the cerate solution to the pale blue end point. Determine a blank titration correction on a similar sample minus the oxalate solution. The milliliters of oxalate used divided by the corrected milliliters of cerate and times 0.01 provide the normality of the cerate. Do not attempt to adjust the solution to exactly 0.01 *N*, and restandardize whenever the reagent is used several days or more apart. Keep in a dark bottle away from light.

H. Nitro-ferroin indicator (nitro-orthophenanthroline ferrous sulfate). Dilute the stock 0.025 *M* indicator solution 1 to 20. Use 0.1 ml. in analyses and 0.2 ml. in standardizations.

Procedure. Pipet an aliquot containing 0.005 to 0.08 m. e. of calcium into a clean 12-ml. conical centrifuge tube, dilute or evaporate to 5 ml., and add 1 drop of (A), 2 drops of (B), and 1 ml. of (C). Heat to the boiling point in a water bath. While twirling the tube, add (D) dropwise until the solution just turns yellow. Replace in the bath, and after 30 minutes cool the tube n air or in water. If necessary add more (D) to keep the solution just yellow.

Centrifuge at 3000 r. p. m. for 10 minutes. Carefully decant the supernatant liquid into a 25-, 50-, or 100-ml. volumetric flask. Stir the precipitate, and rinse the sides of the tube with a stream of 5 ml. of (E) blown from a pipet. Centrifuge at 3000 r. p. m. for 10 minutes. Decant the washings into the same flask. Drain the tube by inversion on filter paper for 10 minutes. Wipe the mouth of the tube with a clean towel or lintless filter paper.

Blow into the tube 3 ml. of (F) from a pipet. When the precipitate is dissolved, add 0.1 ml. of (H). Titrate with (G) from a 10-ml. microburet to the pale blue end point. If more than 5 ml. of (G) is required, transfer the sample to a small beaker and complete the titration. Determine the blank correction in the same manner; it usually is about 0.03 ml. Dilute the supernatant liquids in the volumetric flask to volume and save for the magnesium determination.

Calculation. M. e. of Ca per liter = (corrected ml. of cerate solution × normality of cerate × 1000) ÷ ml. in sample aliquot.

Precision and Accuracy

The calcium concentrations of the seven soil extracts indicated in Table I were determined by this procedure and by a calcium oxalate–potassium permanganate volumetric macromethod outlined by Wilcox (*39*, pp. 38–9) and based on the calcium-magnesium separation technique of Blasdale (*8*).

Table II. Comparison of Macro- and Semimicromethods for Calcium

Soil No.	Macromethod Aliquot	Macromethod Calcium A	Macromethod Calcium B	Macromethod Calcium Mean	Macromethod Deviation	Semimicromethod Aliquot	Semimicromethod Calcium A	Semimicromethod Calcium B	Semimicromethod Calcium Mean	Semimicromethod Deviation	Error
	Ml.	*M. e./liter*			%	*Ml.*	*M. e./liter*			%	%
57	50	39.05	39.09	39.07	0.05	1	39.9	39.9	39.9	0.00	+2.1
62	200	3.53	3.54	3.54	0.14	10	3.57	3.58	3.58	0.14	+1.1
79	200	2.19	2.20	2.20	0.23	10	2.16	2.18	2.17	0.46	−1.4
84	200	2.34	2.34	2.34	0.00	10	2.32	2.34	2.33	0.43	−0.4
85	50	28.35	28.45	28.40	0.18	2	28.6	28.9	28.8	0.52	+1.4
86	50	24.35	24.51	24.43	0.33	2	24.8	24.9	24.9	0.20	+1.9
314	200	7.71	7.80	7.76	0.58	5	7.75	7.80	7.78	0.32	+0.3
				Av.	0.22					0.30	1.2

The results, presented in Table II, indicate highly satisfactory precision and accuracy for the semimicromethod. The reproducibility data demonstrate that it usually is unnecessary to replicate analytical samples.

It has been known that the clinical calcium methods involve a negative error due to loss of calcium oxalate on decanting and a positive error resulting from incomplete washing of the precipitate; Wang (*37*) indicates that these two errors are very evenly balanced in most analyses. The present results support this view and show that the net resultant error is of slight magnitude.

COLORIMETRIC DETERMINATION OF MAGNESIUM

Magnesium is determined on calcium-free solutions by precipitation as magnesium ammonium phosphate hexahydrate, centrifugal washing, and colorimetric estimation of the phosphate content by the ceruleomolybdate reaction. This standard clinical procedure, recently described for plant ash by Wall (*36*), has been modified in some details. No method for the precise determination of small amounts of magnesium in soils appears to have been advanced. Although the method described does not involve a high degree of precision, the use of duplicate analytical samples usually provides satisfactory accuracy.

Because of the sensitivity of the colorimetric phosphate measurement, usually only a fraction of the filtrate from the calcium determination is used for the magnesium determination. This practice is also influenced by the inhibition of precipitation of magnesium by high concentrations of oxalate ion (*14, 24*), which must be reduced to a safe value.

Reagents. The concentrations of molybdate and sulfuric acid used in the development of the blue color are those recommended by Truog and Meyer (*34*), but the strength of the reagent has been modified slightly. The stannous chloride reagent is prepared daily and not acidified, according to Zinzadze (*41*). Because of the effect of time on the color, especially of darker solutions, photometer readings are made at exactly 10 minutes after addition of the stannous chloride.

The ammoniacal wash liquid is similar to that recommended by Wang (*37*) for the washing of calcium oxalate precipitates. Reagents A, B, D, E, and F should be kept in Pyrex bottles and replaced if the precipitation blank color becomes too intense.

A. 30 per cent ammonium chloride solution. Dissolve 30 grams of recrystallized ammonium chloride in water and dilute to 100 ml. Filter before use.

B. 5 per cent ammonium dihydrogen phosphate solution. Dissolve 25 grams of ammonium dihydrogen phosphate in water and dilute to 500 ml. Filter before use.

C. Phenolphthalein, 1 per cent in 60 per cent ethanol.

D. Concentrated ammonium hydroxide.

E. Ammoniacal wash liquid. Mix 20 ml. of concentrated ammonium hydroxide with 80 ml. of water, 100 ml. of ethanol, and 100 ml. of ether.

F. Standard 0.001 *N* magnesium sulfate. This is best prepared by dilution of a more concentrated solution of magnesium sulfate that has been standardized by gravimetric determination of magnesium.

G. 1 *N* sulfuric acid.

H. Ammonium molybdate reagent. Dissolve 40 grams of ammonium molybdate in 400 ml. of water at 60° C., add 456 ml. of arsenic-free concentrated sulfuric acid to 1000 ml. of water, and cool both solutions. Stir the molybdate solution into the acid solution and dilute to 2000 ml. when cool. The reagent is a 2 per cent solution of ammonium molybdate in 8 *N* sulfuric acid. It keeps indefinitely in a brown bottle.

I. Stannous chloride reagent. Place 0.300 gram of c. p. stannous chloride dihydrate in a 100-ml. volumetric flask. Dissolve rapidly in water, dilute to the mark, and mix. Any turbidity will be removed on mixing with reagent H. Prepare fresh daily.

Procedure. From the volumetric flask containing the calcium-free sample pipet an aliquot containing 0.0005 to 0.003 m. e. of magnesium into a 12-ml. conical centrifuge tube and dilute or evaporate to 5 ml. Add 1 ml. each of (A) and (B) and 1 drop of (C). Heat to 90° C. in a water bath and while twirling the tube add (D) dropwise until pink. Cool, add 2 ml. of (D), and stir with a thin glass rod. Withdraw the rod, stopper the tube, and let stand overnight.

Centrifuge at 3000 r. p. m. for 10 minutes, decant carefully, drain on filter paper for 10 minutes, and wipe the mouth of the tube with a clean towel or lintless filter paper. Wash the precipitate and sides of the tube with a stream of 5 ml. of (E) from a pipet equipped with a rubber aspirator bulb or by a similar arrangement. Centrifuge at 3000 r. p. m. for 5 minutes, decant, drain for 5 minutes, and wipe the mouth of the tube. Repeat this washing procedure once.

Pipet 2 ml. of (G) into the tube and dilute to about 10 ml. After 5 minutes, wash the contents into a 100-ml. volumetric flask to which exactly 5 ml. of (H) have previously been added. Dilute to about 60 ml. and pipet in 1 ml. of (I) while rapidly twirling the flask. Dilute to the mark and mix. At exactly 10 minutes after adding (I) measure the light transmission of the blue solution in a photometer test tube through the 650-millimicron filter versus that of water in a similar tube. Previously, the photometer is balanced at 100 per cent transmission with water in both tubes. The accuracy is increased somewhat by the use of the same test tube for all samples and standards.

Prepare a photometer calibration curve on semilogarithmic graph paper by taking a series of 0, 0.5, 1, 2, and 3 ml. of (F) through the same entire procedure. A typical calibration is shown in Figure 1. The amount of magnesium in the sample is obtained by simple interpolation on the curve.

Because of the effect of oxalate, the magnesium sample should not represent more than one fifth of the calcium sample. If the magnesium concentration is so low that it cannot be accurately

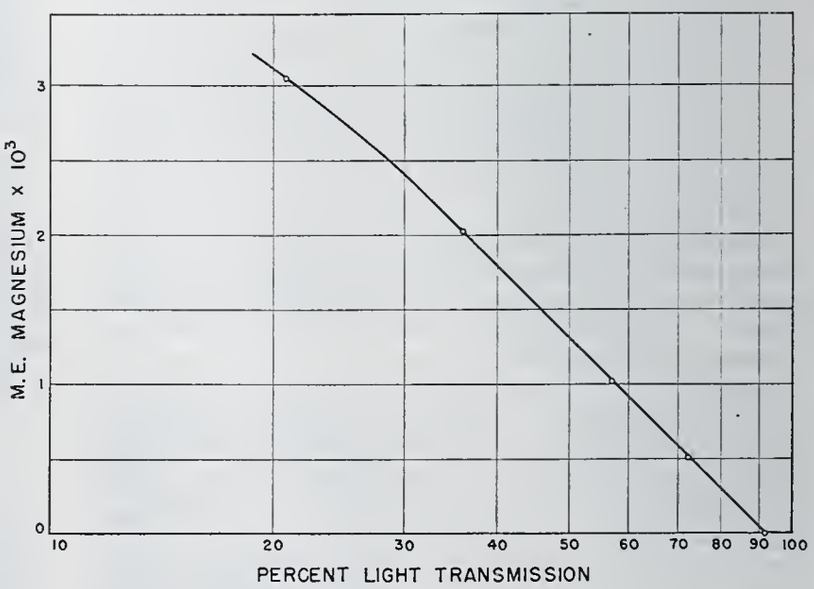

Figure 1. Photometer Calibration Curve for Magnesium

determined in this manner, a larger fraction of the calcium filtrate or even all of it can be used, provided an equivalent amount of oxalic acid is added to each standard sample before precipitation of the magnesium. Results obtained under these conditions should not be expected to be as satisfactory as those obtained by the routine procedure.

CALCULATION. M. e. of Mg per liter = (m. e. of Mg as found by interpolation × 1000) ÷ (ml. in Ca aliquot × fraction of Ca aliquot used for Mg determination).

TABLE III. COMPARISON OF MACRO- AND SEMIMICROMETHODS FOR MAGNESIUM

Soil No.	Macromethod Aliquot	Macromethod Magnesium A	Macromethod Magnesium B	Macromethod Magnesium Mean	Macromethod Deviation	Semimicromethod Aliquot	Semimicromethod Magnesium A	Semimicromethod Magnesium B	Semimicromethod Magnesium C	Semimicromethod Magnesium D	Semimicromethod Magnesium Mean	Semimicromethod Deviation	Error
	Ml.	*M. e./liter*			%	*Ml.*	*M. e./liter*					%	%
57	50	13.34	13.36	13.35	0.08	0.2	12.5	13.1	13.2	13.4	13.1	1.91	−1.9
62	200	3.33	3.35	3.34	0.30	0.8	3.41	3.46	3.20	3.38	3.36	2.46	+0.6
79	200	1.08	1.09	1.09	0.46	1.6	1.11	1.11	1.12	1.11	1.11	0.23	+1.8
84	200	0.58	0.58	0.58	0.00	2	0.58	0.60	0.56	0.57	0.58	2.16	0.0
85	50	12.46	12.50	12.48	0.16	0.2	12.9	12.9	12.3	12.7	12.7	1.57	+1.8
86	50	32.48	32.82	32.65	0.52	0.1	32.1	32.7	32.1	32.5	32.4	0.77	−0.8
314	200	6.33	6.38	6.36	0.39	0.4	6.10	6.48	6.50	6.45	6.38	2.24	+0.3
				Av.	0.27							1.62	1.0

Precision and Accuracy

The magnesium concentrations of the seven soil extracts were determined by this procedure and by a gravimetric method described by Wilcox (*39*, pp. 39–40), except that the magnesium ammonium phosphate hexahydrate precipitates were collected on porous-bottomed ceramic Gooch crucibles, washed with ammonium hydroxide, ethanol, and ether, and weighed as the hexahydrate. Colorimetric magnesium determinations were made on duplicate fractions of each of the two calcium-free semimicroanalytical samples. This arrangement provides an opportunity to decide whether errors possibly arising in the separation of magnesium from calcium contribute significantly to the over-all errors. In Table III, colorimetric samples A and B are from one calcium-free aliquot, and C and D from the other.

The precision of the colorimetric method for most samples as shown by variations among four replicates is not very high. However, the use of average values from duplicate aliquots usually results in acceptable accuracy. Four extracts indicate that errors in the separation of calcium may influence the results, but this is hardly significant and is not upheld by previous data. The interference of other ions, such as sodium and potassium, is always possible when a double precipitation is not made. Hillebrand and Lundell (*14*) discuss these and other interferences in detail. Aside from the various theoretical aspects of the method, it provides satisfactory results if too much faith is not placed in a single determination.

COLORIMETRIC DETERMINATION OF SODIUM

Sodium is determined by a procedure which follows closely the clinical method of Hoffman and Osgood (*17*). Sodium uranyl zinc acetate is precipitated, centrifugally washed, and dissolved, and the yellow color is compared with those of sodium standards. Dissolving the precipitates in ammonium thiocyanate solution helps to stabilize the color against temperature changes. Because of the great sensitivity of the gravimetric uranyl zinc acetate method, the colorimetric technique sometimes may not extend the analytical range to the same extent as for the determination of some other ions. Nevertheless, the photometric method does increase the precision of estimation of small quantities of sodium.

REAGENTS. A. Sodium uranyl zinc acetate crystals. Add 125 ml. of (B) to 5 ml. of 2 per cent sodium chloride solution, stir, and after 15 minutes collect the precipitate in a porous-bottomed porcelain crucible. Wash with several portions of glacial acetic acid, then likewise with ether. Dry in a desiccator over calcium chloride for one hour.

B. Uranyl zinc acetate reagent. *Solution 1.* Stir 80 grams of uranyl acetate dihydrate into a mixture of 14 ml. of glacial acetic acid and 427 ml. of water.

Solution 2. Stir 220 grams of zinc acetate dihydrate into a mixture of 7 ml. of glacial acetic acid and 294 ml. of water.

Heat the two solutions separately on a water bath and stir until the salts are dissolved. Mix while hot, and when cool add 0.2 gram of (A). Let stand overnight. Keep in a dark bottle and filter before use. [For preparation of (A), this reagent does not have to be saturated with (A).]

C. Acetic acid–ethanol wash liquid. Mix 75 ml. of glacial acetic acid with 425 ml. of 95 per cent ethanol. Shake with an excess of (A). Keep in a dark bottle and filter before use.

D. 0.1 *N* ammonium thiocyanate. Dissolve 3.81 grams of C. P. ammonium thiocyanate in water and dilute to 500 ml. Prepare a sufficient quantity fresh each time.

E. Ether, C. P., anhydrous.

F. Standard 0.005 *N* sodium chloride. Dissolve 0.2923 grams of dry recrystallized sodium chloride in water and dilute to exactly 1 liter in a volumetric flask.

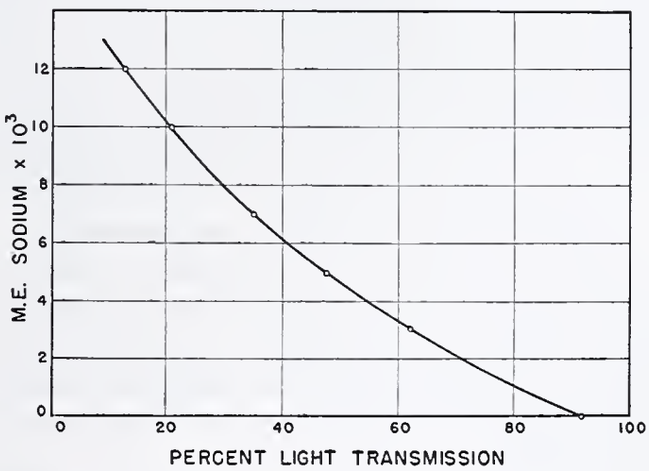

FIGURE 2. PHOTOMETER CALIBRATION CURVE FOR SODIUM

PROCEDURE. Pipet an aliquot containing 0.002 to 0.012 m. e. of sodium into a clean 12-ml. conical centrifuge tube. Evaporate in a water bath to 0.2 ml. Cool, add 8 ml. of (B), stopper, and mix immediately by repeated inversions for one minute. Let stand one hour. Remove the stopper and centrifuge at 3000 r. p. m. for 10 minutes. Drain on filter paper for 10 minutes. Wipe the mouth of the tube with a clean towel or lintless filter paper. Stir the precipitate and wash the sides of the tube with 4 ml. of (C) in a stream from a pipet equipped with a rubber aspirator bulb. Centrifuge at 3000 r. p. m. for 10 minutes, decant, and drain for 10 minutes. Wipe the mouth of the tube. Wash with 5 ml. of ether, but centrifuge for only 5 minutes and decant carefully without draining. (If the tube is drained after the ether wash, portions of a precipitate may drop from the tube.) Repeat the ether washing and decanting once.

When the ether is completely evaporated, pipet into the tube exactly 10 ml. of (D), mix by inversion until the precipitate is dissolved, and centrifuge at 3000 r. p. m. for 5 minutes to remove any phosphate precipitate. Pour into a 1-inch optical cell and measure the light transmission of the solution through the 420-millimicron filter versus that of (D) in a similar cell. Previously, balance the photometer at 100 per cent transmission with (D) in both cells. A calibration curve is prepared by taking a series of 0, 0.5, 1, 1.5, 2, and 2.5 ml. of (F) through the same procedure and plotting the results on ordinary graph paper. Figure 2

shows a typical curve. The amount of sodium in the sample is obtained by simple interpolation on the curve.

Calculation. M. e. of Na per liter = (m. e. of Na as found by interpolation × 1000) ÷ ml. in aliquot.

Precision and Accuracy

The macromethod used for comparison was the original gravimetric uranyl zinc acetate method of Barber and Kolthoff (*6*) as modified by Wilcox [*39*, Sect. 70 (b), p. 42]. The results are presented in Table IV.

Table IV. Comparison of Macro- and Semimicromethods for Sodium

Soil No.	Macromethod Aliquot	Macromethod Sodium A	Macromethod Sodium B	Macromethod Sodium Mean	Macromethod Deviation	Semimicromethod Aliquot	Semimicromethod Sodium A	Semimicromethod Sodium B	Semimicromethod Sodium Mean	Semimicromethod Deviation	Error
	Ml.	*M. e./liter*			%	*Ml.*	*M. e./liter*			%	%
57	5	29.71	29.83	29.77	0.20	0.3	30.0	30.3	30.2	0.50	+1.4
62	2	86.15	86.35	86.25	0.12	0.1	86.5	87.5	87.0	0.58	+0.9
79	50	3.40	3.44	3.42	0.59	2	3.47	3.57	3.52	1.42	+2.9
84	50	5.16	5.16	5.16	0.00	2	5.05	5.15	5.10	0.98	−1.2
85	50	3.48	3.48	3.48	0.00	2	3.53	3.63	3.58	1.40	+2.9
86	10	21.71	21.71	21.71	0.00	0.4	21.3	21.3	21.3	0.00	−1.9
314	5	52.84	53.02	52.93	0.17	0.2	52.5	53.0	52.8	0.47	−0.2
				Av.	0.15					0.76	1.6

The colorimetric results are similar to those found for magnesium in that while the precision of the method is not great the accuracy obtained by using duplicate samples is satisfactory. Since the precipitation procedures of the colorimetric and gravimetric methods are very similar, the source of any errors must lie mainly in the centrifuge or color-measuring technique. Hoffman and Osgood (*17*) point out that the color intensity is affected appreciably by acid, which must be carefully washed out of the centrifuge tube by the ether. Also, the low sensitivity of photometers and colorimeters to yellow colors tends to reduce the attainable precision.

COLORIMETRIC DETERMINATION OF POTASSIUM

Potassium is determined by a photometric method in which the cobalt content of the centrifuged cobaltinitrite precipitate is estimated by treatment with ferrocyanide and choline hydrochloride, according to a method of Jacobs and Hoffman (*18*), later modified for the photoelectric colorimeter by Hoffman (*16*). Morris and Gerdel (*22*) describe a similar method for plant samples.

The technique of precipitation of the potassium sodium cobaltinitrite involves some suggestions of Volk (*35*) applied to the clinical method of Kramer and Tisdall (*21*). Provision is included for the volatilization of ammonia from samples containing amounts that would cause positive errors in the potassium value.

Reagents. A. 30 per cent sodium cobaltinitrite reagent. Dissolve 30 grams of c. p. sodium cobaltinitrite in water, add 2 ml. of glacial acetic acid, and dilute to 100 ml. Prepare a sufficient quantity fresh daily. Filter before use.

B. 70 per cent ethanol.

C. 0.4 per cent choline hydrochloride. Recrystallize choline hydrochloride as follows: Dissolve in a minimum quantity of absolute ethanol, filter, and precipitate by addition of excess ether. Collect on a suction funnel, wash with ether, and dry in a desiccator. Dissolve 0.2 gram in 50 ml. of water. Prepare a sufficient quantity of the solution fresh daily.

D. 0.8 per cent potassium ferrocyanide. Recrystallize potassium ferrocyanide from a boiling saturated aqueous solution by cooling. Collect on a suction funnel and pull air through until dry. Dissolve 0.4 gram in 50 ml. of water. Prepare a sufficient fresh quantity of the solution daily.

E. Standard 0.01 *N* potassium chloride solution. Dissolve 0.7456 gram of dry recrystallized potassium chloride in water and dilute to exactly 1 liter.

F. 1 *N* sodium hydroxide.

G. 1 *N* acetic acid.

H. Methyl orange, 0.01 per cent in water.

Procedure. Pipet an aliquot containing 0.0005 to 0.005 m. e. of potassium into a clean 12-ml. graduated conical centrifuge tube, dilute or evaporate to 1 ml., blow in 2 ml. of (A) from a pipet, and twirl the tube for a few seconds. Let stand 3 hours in a refrigerator at about 5° C., and mix by twirling several times during this interval. Wash the upper walls of the tube with a stream of 0.5 ml. of water, but do not mix it with the precipitation liquid.

Centrifuge at 3000 r. p. m. for 10 minutes and drain on filter paper for 5 minutes. Wipe the mouth of the tube with a clean towel or lintless filter paper. Stir the precipitate and wash the tube walls with a stream of 3 ml. of (B). Centrifuge for 5 minutes and drain. Repeat this washing once. (Ammonia should be absent from the atmosphere during the preceding operations.)

Vigorously stir the precipitate with a stream of 5 ml. of water from a pipet. Immediately place the tube in a boiling water bath and keep it there until the precipitate is completely dissolved. Cool, add exactly 2 ml. of (C), and then blow in exactly 2 ml. of (D). Dilute to the 12-ml. mark and mix by inversion. If turbid, centrifuge for 5 minutes. After an interval of 15 to 30 minutes, compare the light transmission in a 1-inch optical cell through the 580-millimicron filter with that of water in a similar cell.

Previously, balance the photometer at 100 per cent transmission with water in both cells.

Prepare a calibration curve for each set of samples by carrying a series of 0, 0.1, 0.2, 0.3, 0.4, and 0.5 ml. of 0.01 *N* potassium chloride through the same operations. The amount of potassium in the sample is found by interpolation on this curve. A straight-line semilogarithmic calibration curve is obtained for the range 0 to 0.005 m. e. of potassium, as shown in Figure 3.

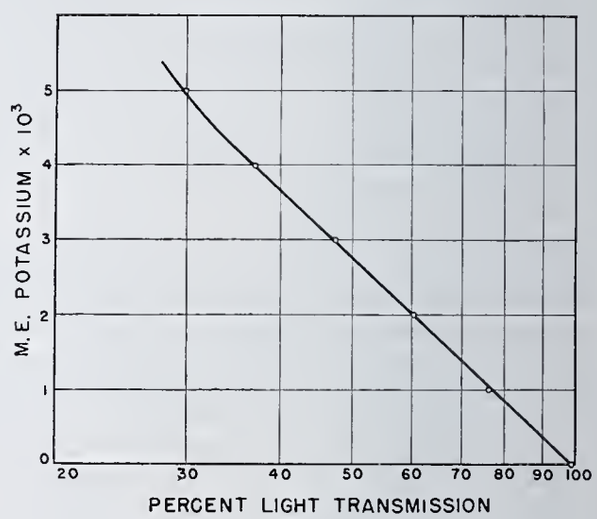

Figure 3. Photometer Calibration Curve for Potassium

If a qualitative or quantitative test for ammonia indicates sufficient to interfere, it can be removed by the following preliminary treatment. Pipet the potassium aliquot into a 15-ml. beaker, dilute to 5 ml., and add 0.2 ml. of (F). Boil slowly until just dry, add 5 ml. of water, and boil again until the residue is barely moist. Dissolve in a minimum volume of water, not over 0.5 ml. and decant into a graduated 12-ml. centrifuge tube. Rinse the beaker with several 0.2-ml. portions of water, and add these to the centrifuge tube. Add 1 drop of (H) and neutralize with (G). Evaporate in a water bath to a volume of 1 ml. Cool and proceed in the regular manner. The series of standards should be treated in the same manner because of the possible addition of potassium by way of the extra reagents.

Calculation. M. e. of K per liter = (m. e. of K in aliquot as found by interpolation × 1000) ÷ ml. in aliquot.

Precision and Accuracy

Quantitative comparisons with macromethods are presented in Table V. Samples 62. 85, and 314 were analyzed

by the gravimetric cobaltinitrite method of Wilcox (*40*). The remainder were analyzed by a volumetric cobaltinitrite procedure, in which the potassium was precipitated according to Wilcox (*40*) and filtered and titrated according to Hibbard and Stout (*13*).

None of the three methods shows a high degree of precision on these samples. Cobaltinitrite methods usually are not the most accurate methods for potassium, but are widely used in soil analysis because of their convenience and sensitivity. The precipitation in both the macro- and semimicroprocedures is probably affected by the same factors. Interferences in saline soils may occur because of the low proportion of potassium to other ions, such as sodium and calcium. In gypsiferous soils, the deposition of calcium sulfate sometimes occurs on evaporation, and the double salt $CaSO_4 \cdot K_2SO_4$ may be formed. The predictable accuracy of the colorimetric method is very satisfactory when compared to the macroprocedures.

COLORIMETRIC DETERMINATION OF AMMONIUM

In samples containing appreciable quantities of ammonium ion resulting from ammonification, fertilization, or other source, it may be desirable to determine its concentration. This can be accomplished by the procedure outlined under the description of the colorimetric determination of nitrate.

Table V. Comparison of Macro- and Semimicromethods for Potassium

Soil No.	Macromethod Aliquot	Macromethod Potassium A	Macromethod Potassium B	Macromethod Potassium Mean	Macromethod Deviation	Semimicromethod Aliquot	Semimicromethod Potassium A	Semimicromethod Potassium B	Semimicromethod Potassium Mean	Semimicromethod Dviation	Error
	Ml.	*M. e./liter*			%	*Ml.*	*M. e./liter*			%	%
57	200	0.417	0.425	0.421	0.95	10	0.428	0.437	0.433	1.04	+2.9
62	100	3.10	3.16	3.13	0.96	1	3.18	3.22	3.20	0.63	+2.2
79	100	0.704	0.704	0.704	0.00	5	0.673	0.677	0.675	0.30	−4.1
84	100	0.335	0.339	0.337	0.59	10	0.340	0.346	0.343	0.87	+1.8
85	100	3.56	3.61	3.59	0.70	1	3.53	3.60	3.57	0.98	−0.6
86	100	1.086	1.106	1.096	0.91	5	1.114	1.128	1.121	0.62	+2.3
314	100	0.98	1.02	1.00	2.00	5	0.98	1.00	0.99	1.01	−1.0
				Av.	0.87					0.78	2.1

VOLUMETRIC DETERMINATION OF CARBONATE AND BICARBONATE

These ions are determined by a micromodification of the Warder (*38*) alkalimetric titration to the phenolphthalein and methyl orange end points, respectively. Titration methods are rapid in operation, but can be highly inaccurate under the ordinary conditions of sampling and analysis. These factors were stressed by Johnston (*19*). More recently, Benedetti-Pichler *et al.* (*7*) discussed the various errors of the ordinary Warder procedure. The estimation of a small amount of carbonate in the presence of a much larger quantity of bicarbonate, a condition frequently encountered in alkaline soil extracts, usually involves considerable error. [Hirsch (*15*) recently described the construction of a slide rule for the calculation of these ions from the pH value and the methyl orange alkalinity. His charts, based on earlier calculations involving carbonate-bicarbonate equilibria, show that no titration method, potentiometric or colorimetric, can be theoretically exact. The use of such a slide rule should provide more accurate results than those obtained from the usual calculation, especially where the carbonate value represents a small fraction of the total alkalinity.] Additional errors may result from the presence of other titratable anions, such as silicate, phosphate, and borate (*2*).

In spite of these difficulties, no other method approaches it in convenience. The indicated method of calculation is the customary one and assumes the absence of other titratable ions.

Reagents. A. Phenolphthalein, 1 per cent in 60 per cent ethanol. B. Methyl orange, 0.01 per cent in water. C. Standard 0.01 *N* sulfuric acid.

Procedure. Pipet an aliquot containing 0.005 to 0.04 m. e. of chloride into a 15-ml. wide-mouthed porcelain crucible or a small porcelain casserole. Add one drop of (A). If the solution turns pink, add (C) from a 10-ml. microburet dropwise at 5-second intervals until the color just disappears. Record the buret reading. Add 2 drops of (B) and titrate to the first orange color. Save the titrated sample for the chloride determination.

An indicator correction blank in boiled water should be determined, and applied if it is not negligible. The lighting should be adequate for the recognition of the various colors. The use of comparison color standards at the correct end points is helpful.

Calculation. If A is the milliliters of (C) to the phenolphthalein end point and B the milliliters to the methyl orange end point,

M. e. of CO_3^{--} per liter = $(2A \times 0.01 \times 1000) \div$ ml. in aliquot.
M. e. of HCO_3^- per liter = $[(B - 2A) \times 0.01 \times 1000] \div$ ml. in aliquot.

Precision and Accuracy

A similar method employing 0.05 *N* sulfuric acid was used for comparison (*5*, p. 535; *39*, p. 18). The results on the seven extracts are presented in Table VI. As the samples were neutral to phenolphthalein, the listed bicarbonate values consist of methyl orange alkalinities.

The precision of the semimicromethod corresponds favorably to that of the official method. This should be expected of a method involving merely a straightforward titration. It is somewhat more difficult to estimate the accuracy of the method; although by comparison with the official method it is satisfactory, the various factors previously discussed affect both methods. Comparisons made on other samples by potentiometric and indicator procedures, not reported here, provided similar accuracy.

VOLUMETRIC DETERMINATION OF CHLORIDE

Chloride is determined by a micromodification of Mohr's volumetric silver nitrate–potassium chromate method (*33*). The determination is made on the carbonate-bicarbonate sample, which has been neutralized to methyl orange in that

Table VI. Comparison of Macro- and Semimicromethods for Bicarbonate

Soil No.	Macromethod Aliquot	Macromethod Bicarbonate A	Macromethod Bicarbonate B	Macromethod Bicarbonate Mean	Macromethod Deviation	Semimicromethod Aliquot	Semimicromethod Bicarbonate A	Semimicromethod Bicarbonate B	Semimicromethod Bicarbonate Mean	Semimicromethod Deviation	Error
	Ml.	*M. e./liter*			%	*Ml.*	*M. e./liter*			%	%
57	100	0.38	0.39	0.39	1.28	10	0.37	0.37	0.37	0.00	−5.1
62	100	1.73	1.73	1.73	0.00	10	1.76	1.78	1.77	0.57	+2.3
79	100	2.67	2.69	2.68	0.37	10	2.67	2.70	2.69	0.56	+0.4
84	100	2.29	2.32	2.31	0.65	10	2.27	2.27	2.27	0.00	−1.7
85	100	1.80	1.81	1.81	0.28	10	1.79	1.81	1.80	0.56	−0.6
86	100	2.85	2.86	2.86	0.17	10	2.78	2.81	2.80	0.54	−2.1
314	50	1.07	1.08	1.08	0.46	10	1.11	1.13	1.12	0.89	+3.7
				Av.	0.46					0.45	2.3

procedure. With adequate lighting and appropriate blank corrections, satisfactory accuracy can be obtained.

Reagents. A. 5 per cent potassium chromate indicator. Dissolve 5 grams of potassium chromate in 50 ml. of water and add 1 *N* silver nitrate dropwise until a slight permanent red precipitate is produced. Filter and dilute to 100 ml.

B. Standard 0.005 *N* silver nitrate solution. Dissolve 0.8495 gram of c. p. silver nitrate in water and dilute to exactly 1 liter. Keep in a brown bottle away from light.

Procedure. To the sample preserved from the carbonate-bicarbonate determination, add 4 drops of (A). While stirring, titrate under a bright light with (B) from a 10-ml. microburet to the first permanent light brown color. The titration blank correction varies with the volume of the sample at the end point, and usually increases regularly from about 0.03 to 0.20 ml. as the volume increases from 2 to 12 ml.

Calculation. M. e. of Cl^- per liter = (ml. of $AgNO_3$ − ml. of $AgNO_3$ for blank) × 0.005 × 1000 ÷ ml. in aliquot.

Table VII. Comparison of Macro- and Semimicromethods for Chloride

Soil No.	Macromethod Aliquot	Macromethod Chloride A	Macromethod Chloride B	Macromethod Chloride Mean	Macromethod Deviation	Semimicromethod Aliquot	Semimicromethod Chloride A	Semimicromethod Chloride B	Semimicromethod Chloride Mean	Semimicromethod Deviation	Error
	Ml.	*M. e./liter*			%	*Ml.*	*M. e./liter*			%	%
57	20	63.3	63.5	63.4	0.16	0.5	63.4	63.6	63.5	0.16	+0.2
62	20	80.2	80.3	80.3	0.06	0.5	80.4	80.5	80.5	0.06	+0.2
79	100	1.30	1.30	1.30	0.00	10	1.25	1.26	1.26	0.40	−3.1
84	100	1.04	1.05	1.05	0.48	10	0.99	1.00	1.00	0.50	−4.8
85	100	1.52	1.52	1.52	0.00	10	1.49	1.51	1.50	0.67	−1.3
86	100	1.77	1.78	1.78	0.28	10	1.76	1.79	1.78	0.84	0.0
314	50	17.93	17.94	17.94	0.03	2	17.93	17.98	17.96	0.14	+0.1
				Av.	0.14					0.40	1.4

Precision and Accuracy

The seven soil extracts were analyzed by this procedure and by the official Mohr method (*5*, p. 528) as outlined by Wilcox (*39*, p. 18). The results are presented in Table VII. The reproducibility of the semimicromethod is seen to be very satisfactory, comparable to that of the macromethod. Both the precision and accuracy improve with increasing quantities of chloride.

The end point of the chloride titration sometimes is not very distinct, which is probably the main source of error. Because of the low bicarbonate content of several extracts, the aliquots used for the bicarbonate determination were larger than those for the chloride determination.

VOLUMETRIC DETERMINATION OF SULFATE

Sulfate is determined by precipitation as benzidine sulfate, centrifugation, and direct titration of the liberated sulfuric acid with dilute standard base. The reagents and precipitation procedure are based on Fiske's modification (*11*) of the Rosenheim-Drummond method (*29*). Precipitation in an ice bath and centrifugal washing appear to increase the accuracy. A source of error in benzidine methods has been the coprecipitation of benzidine hydrochloride, which is difficult to wash out of the precipitate (*12*). It is believed that the method described here reduces this error.

Reagents. A. Bromophenol blue, 0.04 per cent in 95 per cent ethanol.

B. 1 *N* hydrochloric acid.

C. Benzidine hydrochloride reagent. Purify benzidine hydrochloride and prepare the reagent as follows: Dissolve 10 grams of benzidine hydrochloride in 400 ml. of 1 *N* hydrochloric acid by warming to 50° C. Filter, add 40 ml. of concentrated hydrochloric acid with stirring, cool in ice water for 30 minutes, and collect crystals on a Büchner funnel. Wash with cold 1 *N* hydrochloric acid, then with two 25-ml. portions of cold 95 per cent ethanol and four portions of ether. When dry, transfer to a brown bottle. Suspend 4 grams in 150 ml. of water in a 250-ml. volumetric flask, add 7 ml. of 1 *N* hydrochloric acid, shake until dissolved, and dilute to the mark. Filter before use.

D. 95 per cent acetone.

E. Phenol red. Prepare a 0.05 per cent aqueous solution. To reduce the titration blank caused by the indicator, add sufficient 0.05 *N* sodium hydroxide so that when diluted in the titration sample, the blank will be no greater than 0.05 ml. of 0.01 *N* sodium hydroxide. As an illustration, 3 parts of phenol red solution are treated with 1 part of 0.05 *N* sodium hydroxide, when 0.2 ml. of indicator is used in the titration of unknowns and standard phthalate.

F. Standard 0.01 *N* sodium hydroxide. This should be standardized against 5 ml. of 0.01 *N* potassium acid phthalate at the boiling point with phenol red. The end point is the deep purplish-red color that persists on boiling. The total volume of titrated sample at the end point should be 10 ml. Do not attempt to make exactly 0.01 *N*.

Procedure. Pipet an aliquot containing 0.005 to 0.08 m. e. of sulfate into a clean 12-ml. conical centrifuge tube. Dilute or evaporate to 5 ml. Add 2 drops of (A) and then (B) dropwise until yellow. Place in ice water, and after 5 minutes blow in 2 ml. of (C) from a pipet, and mix well by twirling. Let stand 20 minutes in ice water, and centrifuge at 3000 r. p. m. for 10 minutes. Decant carefully without draining. Wash the tube walls and stir the precipitate with a stream of 5 ml. of (D) from a pipet. Centrifuge at 3000 r. p. m. for 5 minutes, decant, and wash twice again in the same manner.

Wash the precipitate into a 50- or 100-ml. beaker with a 10-ml. stream of water, add 0.2 ml. of (E), and titrate boiling hot with (F) from a 10-ml. microburet. During the titration, pour the hot solution back and forth from the beaker to the centrifuge tube, to remove any adhering precipitate. Titrate to the same permanent end-point tint used in the standardization. The total volume of titrated sample at the end point should be 10 ml. Determine the titration blank by exactly the same procedure.

Calculation. M.e. of SO_4 per liter = (ml. of NaOH − ml. of NaOH for blank) × normality of NaOH × 1000 ÷ ml. in aliquot.

Precision and Accuracy

A gravimetric barium sulfate method (*39*, p. 19) was used for comparison. Table VIII contains the results. The semimicromethod shows a high degree of precision and a predictable accuracy of about 2 per cent.

It is important that all titrations, including unknowns, blanks, and standardizations, be made to the same phenol red end point in equal final volumes of sample. Precipitation in an acid solution precludes the interfering precipitation of phosphate.

COLORIMETRIC DETERMINATION OF NITRATE

Nitrate is determined by a distillation method, because the well-known phenoldisulfonic acid method is seriously affected by chloride. The procedure involves reduction of nitrate to ammonia, distillation into dilute acid, nesslerization, and photometric comparison with standards similarly treated. Any nitrite is included in the nitrate value. Ammonium ion can be determined by preliminary distillation in the absence of Devarda's alloy.

Various nitrogenous organic compounds are hydrolyzed on boiling with sodium hydroxide, sodium carbonate, and magnesium oxide with the formation of ammonia (*23*, *25*, *31*). If ammonia is distilled prior to the nitrate reduction, possible errors arising from the hydrolysis of such compounds in soil solutions or extracts are included in the ammonium value. According to Nichols and Foote (*23*) and Shrikhande (*31*), this error in the estimation of free ammonia can be eliminated by distilling the ammonia from a solution buffered at pH 7.4.

Table VIII. Comparison of Macro- and Semimicromethods for Sulfate

Soil No.	Macromethod Aliquot	Sulfate A	Sulfate B	Sulfate Mean	Deviation	Semimicromethod Aliquot	Sulfate A	Sulfate B	Sulfate Mean	Deviation	Error
	Ml.	*M. e./liter*			%	*Ml.*	*M. e./liter*			%	%
57	100	14.85	14.94	14.90	0.30	3	15.0	15.0	15.0	0.00	+0.7
62	100	13.76	13.78	13.77	0.07	3	14.0	14.1	14.1	0.35	+2.4
79	200	2.49	2.49	2.49	0.00	10	2.40	2.45	2.43	1.03	−2.4
84	200	4.38	4.39	4.39	0.11	10	4.50	4.53	4.52	0.33	+3.0
85	50	41.54	41.60	41.57	0.07	1	42.5	42.6	42.6	0.12	+2.5
86	50	71.70	71.80	71.75	0.07	1	72.9	73.0	73.0	0.07	+1.7
314	50	47.58	47.90	47.74	0.34	1	48.5	48.8	48.7	0.31	+2.0
				Av.	0.14					0.32	2.1

The distillation equipment includes microburners, 100-ml. Kjeldahl flasks, and "inverted U" air condensers of 1.9-cm. (0.75-inch) diameter. (The glassware is obtainable from the Hengar Company, Philadelphia, Penna.) A distillation rack of six units is convenient. The Nessler reagent is prepared according to Koch and McKeekin (*20*). The distilled water should be practically free of nitrogen compounds.

Reagents. A. Devarda's alloy. Boil a quantity in 0.2 *N* sodium hydroxide for a few minutes to reduce the nitrogen content. Wash and dry.

B. 2 *N* sodium hydroxide. Dissolve 80 grams of nitrogen-free sodium hydroxide in 1 liter of water.

C. 0.01 *N* sulfuric acid.

D. Standard 0.01 *N* potassium nitrate. Dissolve 1.011 grams of dry recrystallized potassium nitrate in water and dilute to exactly 1 liter.

E. Standard 0.01 *N* ammonium sulfate. Dissolve 0.6607 gram of pyridine-free ammonium sulfate in water and dilute to exactly 1 liter.

F. Nessler reagent (*19*). "Dissolve 22.5 grams of iodine in 20 cc. of water containing 30 grams of potassium iodide. After the solution is completed, add 30 grams of pure metallic mercury, and shake the mixture well, keeping it from becoming hot by immersing in tap water from time to time. Continue this until the supernatant liquid has lost all of the yellow color due to iodine. Decant the supernatant aqueous solution and test a portion by adding a few drops thereof to 1 cc. of a 1 per cent soluble starch solution. Unless the starch test for iodine is obtained the solution may contain mercurous compounds. To the remaining solution add a few drops of an iodine solution of the same concentration as employed above, until a faint excess of free iodine can be detected by adding a few drops thereof to 1 cc. of the starch solution. Dilute to 200 cc. and mix well. To 975 cc. of an accurately prepared 10 per cent sodium hydroxide solution now add the entire solution of potassium mercuric iodide prepared above. Mix thoroughly and allow to clear by standing." Keep in a brown bottle.

Procedure. Pipet an aliquot containing 0.002 to 0.015 m. e. of nitrate into a 100-ml. Kjeldahl flask. (If the sample contains carbonate, barely neutralize the aliquot with dilute sulfuric acid.) Add 0.50 gram of (A) and dilute to 30 ml. Immerse the tip of the delivery tube in a mixture of 3 ml. of (C) and 20 ml. of water in a 100-ml. beaker.

Carefully run 2 ml of (B) down the neck of the flask. Connect the flask to the air condenser by the rubber sleeve and twirl it, to mix the contents. Heat the flask with a microburner until 15 ml. have been distilled. Raise the distillation assembly free of the acid and distill one minute longer. Disconnect the delivery tube and rinse it into the beaker. Wash the contents of the beaker into a 100-ml. volumetric flask, add exactly 5 ml. of (F) while whirling the flask, dilute to the mark, and mix. After 15 minutes, measure the light transmission through the 460-millimicron filter in a 1-inch optical cell against that of water in a similar cell. Previously balance the photometer at 100 per cent transmission with water in both cells.

Distill and treat a series of 0, 0.2, 0.5, 1, and 1.5 ml. of (D) in the same manner. From the photometer readings plot a calibration curve on semilogarithmic graph paper. The milliequivalents of nitrate in the aliquot are determined by interpolation on this curve. A typical curve is shown in Figure 4.

If a qualitative nesslerization test indicates a measurable amount of ammonia, both ammonium and nitrate can be determined on the same aliquot by the following modification. Distill, nesslerize, and measure light transmission in the manner described, omitting (A) and substituting a boiling stone. Now immerse the tip of the rinsed delivery tube in a fresh mixture of 3 ml. of (C) and 20 ml. of water in a clean beaker; add to the cooled Kjeldahl flask 0.50 gram of (A) and 15 ml. of water. Immediately connect to the condenser, distill, and treat in the same manner.

To obtain the ammonium calibration curve, distill, without Devarda's alloy, a series of 0, 0.2, 0.5, 1, and 1.5 ml. of (E), nesslerize, and measure the light transmission. Prepare the nitrate calibration curve as previously outlined. Determine the milliequivalents of ammonium and nitrate ions in the aliquot by interpolation on their respective curves.

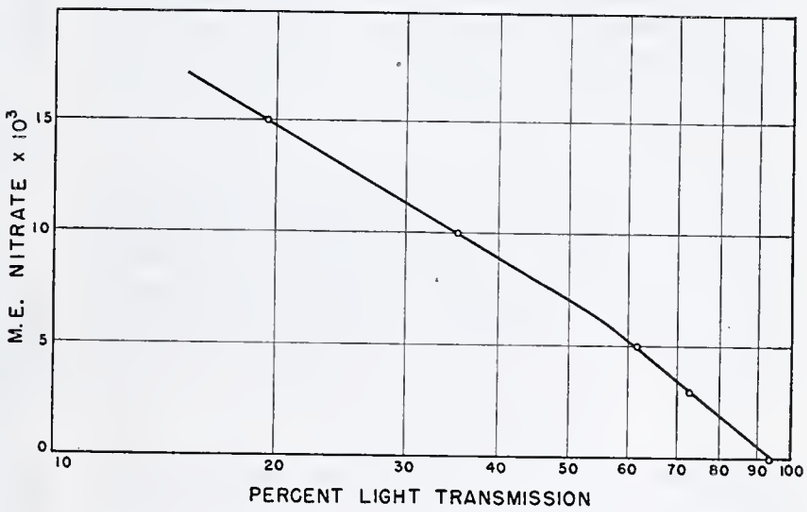

Figure 4. Photometer Calibration Curve for Nitrate

If it is suspected that hydrolyzable nitrogenous organic compounds are increasing the ammonium value, an additional distillation should be made in which the Kjeldahl flask contents are buffered at pH 7.4, according to Shrikhande (*31*). A series of ammonium standards can be treated in the same manner.

Table IX. Comparison of Macro- and Semimicromethods for Nitrate

Soil No.	Macromethod Aliquot	Nitrate A	Nitrate B	Nitrate Mean	Deviation	Semimicromethod Aliquot	Nitrate A	Nitrate B	Nitrate Mean	Deviation	Error
	Ml.	*M. e./liter*			%	*Ml.*	*M. e./liter*			%	%
57	200	3.72	3.73	3.73	0.13	4	3.63	3.63	3.63	0.00	− 2.7
62	400	0.673	0.680	0.677	0.52	10	0.675	0.690	0.683	1.10	+ 0.9
79	400	0.432	0.439	0.436	0.80	10	0.445	0.450	0.448	0.56	+ 2.8
84	400	0.259	0.259	0.259	0.00	20	0.280	0.285	0.283	0.88	+ 9.3
85	400	2.35	2.36	2.36	0.21	5	2.36	2.36	2.36	0.00	0.0
86	400	0.309	0.309	0.309	0.00	30	0.345	0.345	0.345	0.00	+11.6
314	400	0.877	0.879	0.878	0.11	10	0.875	0.895	0.885	1.13	+ 0.8
				Av.	0.25					0.52	4.0

CALCULATION. M. e. of NO_3 per liter = (m. e. of NO_3 in aliquot as found by interpolation on NO_3 curve × 1000) ÷ ml. in aliquot.

M. e. of NH_4 per liter = (m. e. of NH_4 in aliquot as found by interpolation on NH_4 curve × 1000) ÷ ml. in aliquot.

Precision and Accuracy

The seven soil extracts were analyzed for nitrate by this method and by a regular Devarda procedure (*39*, p. 21) in which the ammonia is collected in boric acid solution and titrated with 0.05 *N* sulfuric acid. For this purpose, no preliminary separation of ammonia was made, and the values in Table IX include all nitrogen that would be liberated under the analytical conditions.

The extracts of soils 84 and 86 contained so little nitrate that the results by the titration method are probably inaccurate. By excluding these from the accuracy comparisons, the average "error" of the semimicromethod is reduced from 4.0 to 1.5 per cent. As for some other ions, the semimicromethod sometimes may actually provide more accurate results than the comparison method. The colorimetric method shows a satisfactory degree of precision.

Discussion

The volume of sample used in the semimicroanalysis of the seven soil extracts comprised $^1/_{15}$ to $^1/_{36}$ of that used in the comparison methods, with an average of $^1/_{24}$. This represents a considerable reduction in sample requirements. In general, semimicroanalysis requires less time, although no quantitative comparisons have been made. The economy effected in the analytical reagents is often important.

The accuracy obtainable under these conditions is not seriously reduced, and is adequate for most soil analyses. The average predictable error of the methods is about 2 per cent, based on comparisons with methods involving much larger samples. Many of the methods are sufficiently precise that replication of determinations usually is unnecessary. The quantitative reproducibility data presented for the various methods should assist in determining the desirability of replication, based on particular requirements.

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Estimation of the Sulfonamides

A Rapid and Accurate Micromethod

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RECENT work in the sulfonamide field, in which attempts are being made to correlate low blood levels with efficacy, has made exact determinations of these drugs more important than ever. This need, and the desire to obtain the advantages of working on a micro scale, have led to a method which has most of the advantages of the methods now in wide use, and practically none of their shortcomings.

TABLE I. FREE SULFATHIAZOLE LEVELS

Subject and Sulfathiazole Dose	Regular Bratton and Marshall Method	Micromethod Blood laked before pptn.	Micromethod Blood pptd. directly
	Mg. %	*Mg.* %	*Mg.* %
Rabbit, 0.5 gram orally, blood taken after one hour	3.0 (trip.)	.....	3.5 (trip.)
Man, 2 grams taken orally, blood taken after 2 hours	2.9 (dupl.)	.....	3.6 (dupl.)
Man, 1 gram taken orally, blood taken after 2 hours	2.8	3.3 (dupl.)	3.3 (dupl.)
Rabbit, 0.5 gram taken orally, sample after 1 hour	3.5 (quad.)	4.0 (dupl.)	4.0 (dupl.)
Man, 2 grams orally, sample after 1 hour	2.3 (quad.)	2.6 (quad.)	2.6 (trip.)
Man, 2 grams orally, sample after 4 hours	2.7 (trip.)	3.1 (quad.)	3.1 (quad.)

The methods most commonly used are those of Bratton and Marshall (*2*) and Werner (*8*), or modifications or adaptations of them. In the former procedure, the sulfonamide is diazotized, and the diazonium salt coupled with *N*(1-naphthyl)ethylenediamine dihydrochloride. The azo dye which is formed is determined colorimetrically. The undesirable features of this procedure have been mentioned in a preliminary note on the method under discussion (*5*). In the Werner method, *p*-dimethylaminobenzaldehyde is used to form a yellow anil with the "free" sulfonamides, and the intensity of this color is measured. It is difficult to use this method for accurate micro work because of the low tinctorial value of the yellow dye. It has also been pointed out that "total" values obtained with these methods are subject to error resulting from the change in color intensity with small changes in pH (*6*).

The time required for a single analysis is greatly reduced using the micromethod described below; an analysis may be completed in 7 or 8 minutes. This is even less than the time necessary for the Werner method, which is about 12 minutes (*1*). Reasonable amounts of sodium chloride do not interfere. Fifty mg. per cent or less of potassium thiocyanate (*6*) did not interfere in the determination of 10 mg. per cent of sulfathiazole by the Bratton and Marshall or the micromethod. This concentration of thiocyanate is much higher than is ever obtained in the blood. Blank readings on normal blood (human, horse, and rabbit) were found to be zero as a rule, and never exceeded 0.2 mg. per cent.

Determination of Sulfa Drugs

FREE SULFA DRUGS. In the micromethod, the blood is precipitated directly in a mixture of trichloroacetic and sulfuric acids ("acid mixture"). A small amount of sulfuric acid was found to aid materially in a quick and complete precipitation of the protein, and was present in sufficient amount for hydrolysis purposes, for use in the "total" sulfonamide determinations. Laking the blood prior to precipitation is unnecessary (Table I).

The precipitated protein is filtered off, the diazotization is carried out, ethyl alcohol is added, and the naphthylethylenediamine dihydrochloride is added immediately. The color attains its maximum intensity in 15 seconds. In the presence of alcohol, it is not necessary to destroy the excess nitrous acid, for possible products formed by its reaction with the dye are likely to be of the same color and soluble in the medium. No nitrogen bubbles are formed, because the sulfamate–nitrous acid reaction is eliminated, and little or no interference from bubbles (*7*) was noticed. Parallel experiments showed that the sulfamate addition is not necessary, the colors being even more stable in its absence. The recoveries obtained by different means are shown in Tables I, IV, and V.

In the tables, results are expressed in different terms for the sake of clearness. In some cases milligram per cent figures are given; in others, actual density readings from the drum of the Coleman spectrophotometer are given. Density is defined as the logarithm of the reciprocal of the fraction of transmitted light. If Beer's law is obeyed, as it is in this case, the density is a linear function of the concentration. All results have been checked. Elapsed time was measured with a stop watch. All blood was added at the ratio 1 to 20. All blood samples were whole and oxalated, and usually less than 0.5 hour old. It was found that blood which contained sulfathiazole increased in apparent sulfa drug content by about 10 per cent after standing at room temperature for 20 hours. Appropriate concentrations of trichloroacetic acid were present in all diazotizations. All density readings were made against reagent blanks of the same age.

Accurate analysis was possible for a period of 24 hours. The stability of the colors in the various methods is shown in Table II.

TOTAL SULFA DRUGS. In the determination of total sulfa drug concentration, the Bratton and Marshall method is much to be preferred to the shorter method of Werner (*8*), primarily because of the negligible effect of mineral acids on the intensities of the azo colors formed. In the micromethod 0.1 ml. of 4 *N* sulfuric acid is used in the hydrolysis, although 0.4 ml. of the acid does not seriously alter the density read-

TABLE II. STABILITY OF COLORS FORMED IN VARIOUS METHODS

Method	Density Reading of a 10-μg. Sample of Sulfathiazole after: 5 min.	1 hour	2 hours	3 hours	4 hours	24 hours
Aqueous sulfathiazole solution, Bratton and Marshall procedure	0.25	0.25	0.20	..	..	0.15
Aqueous sulfathiazole solution, micromethod without sulfuric acid	0.25	...	0.255	0.26	0.26	0.26
Aqueous sulfathiazole solution, micromethod	0.25	...	0.26	0.26	0.26	0.25
Sulfathiazole in rabbit blood filtrate, micromethod	0.25	0.245	...	..	..	0.235

Table III. Recoveries of Sulfathiazole Added to Urine

Final Dilution	Recovered by Bratton and Marshall	Recovered by Micromethod
	%	%
1:10	90	90
1:20	92	98
1:30	96	99
1:60	99	100

Table IV. Recoveries of Sulfathiazole from Horse Blood

(Regular procedures as outlined were followed. Sulfathiazole was added so that each 2-ml. aliquot of filtrate should contain 2, 4, 6, 8, or 10 mg. of the drug.)

Method	Sulfathiazole Recovered 2 mg. % added	4 mg. % added	6 mg. % added	8 mg. % added	10 mg. % added
	Milligram per cent				
Bratton and Marshall	1.85	..	5.27	..	8.5
Micromethod	1.86	3.76	5.8	7.75	9.5
Micromethod without sulfuric acid	1.95	4.0	5.8	7.84	9.6

ings. If the acid mixture of trichloroacetic and sulfuric acids is used for precipitation of the blood, the filtrate may be used directly for the total sulfa drug determination.

Sulfa Drugs in Urine. The recovery of sulfathiazole added to various dilutions of normal urine is shown in Table III, compared with results obtained on the same samples by the Bratton and Marshall procedure.

Recovery of Sulfa Drugs from Whole Blood

Much has been written (*7*) about the recovery of sulfa drugs from blood. It is the general opinion that, using the Bratton and Marshall method, added sulfathiazole is recovered from whole blood to the extent of 85 to 90 per cent, at the dilution of 1 to 20. This was confirmed, as shown in Tables IV and V. Work previously reported (*6*) and experiments carried out in this laboratory indicate that the filtrates from precipitated blood (with added sulfathiazole and at 1 to 20 dilution) contain practically all the sulfathiazole (95 to 100 per cent). Low recoveries were obtained with the Bratton and Marshall procedure when the sulfathiazole was added to the whole blood, when whole blood was laked and precipitated in the presence of added sulfathiazole, and in some cases when the sulfathiazole was added to blood filtrates (Tables I, IV, and V). This is contrary, in part, to the findings of Sunderman and Pepper (*7*).

These results indicate that the rates of diazotization in various media are of importance. The diazotization experiments are summarized in Tables VI and VII, which give rates and extents of the various reactions. . These experiments indicate that the variation in recoveries with different drugs, and with different conditions of precipitation (medium, temperature, dilution), is to be explained on the basis of incompleteness of the reactions leading to the formation of the dye.

The diazotizations at 25° C. summarized in Table VI were carried out in a constant-temperature bath, using the procedure indicated. In the Bratton and Marshall reactions 0.10 ml. of freshly prepared 0.100 per cent sodium nitrite was added to 3.00 ml. of solution. In the micromethod the same quantity of nitrite was added to 2.00 ml. of solution, both in the reaction with and without added sulfuric acid. The indicated presence of sodium chloride means that 1.0 ml. of 0.7 per cent saline was added.

The experimental conditions for diazotizations at 35° C. were the same as those in Table IV.

The addition of a small amount of sulfuric acid to the usual trichloroacetic acid decreased the recovery very slightly. The recovery of sulfathiazole added to whole blood, using the micromethod, is almost quantitative (95 to 100 per cent). A comparison of recoveries is given in Table IV.

The variation in recoveries among the sulfa drugs has been explained on a solubility basis: the more insoluble the sulfonamide, the greater the error in the determination due to loss in preparing the blood filtrate (*4*). This explanation seems unlikely when one considers that only micrograms are present in solutions in which tenths or hundredths of grams will dissolve, so that solutions are often one ten-thousandth saturated. The sulfa drugs vary in solubility by factors of 10 or 20. Solubility would thus be expected to exert little influence on the recoveries.

Adsorption of the drug on the precipitated protein can play no important role in producing the low results, for complete (98 to 100 per cent) recovery can be obtained by the micromethod after blood has been precipitated in the presence of the sulfa drug at 1 to 20 dilution. The Bratton and Marshall procedure gives 85 per cent recoveries under these conditions.

On the blood of subjects who received sulfathiazole orally, the micromethod gives results that are about 15 per cent higher than those given by the Bratton and Marshall method. This was taken to indicate complete recovery by the modified method, in conjunction with the results given in Table V. Table I gives typical blood analyses by the two procedures, and shows that laking the blood before precipitation is not necessary.

Table V. Recoveries of Sulfathiazole Added to Various Media

Method	Diazotization Time	Temperature	Medium	Sulfathiazole Added 1	2	Sulfathiazole Found 1	2
	Min.	*° C.*		*Mg. %*		*Mg. %*	
B. & M.	3	ca. 20	Water and CCl_3CO_2H	4	8	3.2	6.0
B. & M. (in presence of NaCl)	3	ca. 20	Water and CCl_3CO-OH	4	8	4.0	8.0
B. & M.	10	ca. 25	Water and CCl_3CO_2H	4	8	4.0	8.0
B. & M.	3	25	Rabbit blood before precipitation	4	8	3.5	6.4
B. & M.	3	25	Sterile, oxalated horse blood before laking, etc.	5	5	3.6	3.7
Micro	3	25	Sterile, oxalated horse blood before laking, etc.	5	5	4.9	4.9
B. & M.	3	25	Filtrate from rabbit blood	4	8	3.6	6.6
B. & M.	4	25	Filtrate from human blood	4	8	3.8	7.1
B. & M.	3	ca. 22	Trichloroacetic acid soln. before addn. and pptn. of rabbit blood	10	10	8	8
B. & M.	10	ca. 22	Trichloroacetic acid soln. before addn. and pptn. of rabbit blood	10	10	9.8	9.9
B. & M.	3	25	Trichloroacetic acid soln. before addn. and pptn. of rabbit blood	4	8	3.7	6.5
B. & M. (in presence of NaCl)	3	25	Trichloroacetic acid soln. before addn. and pptn. of rabbit blood	10	10	9.9	10
B. & M.	3	25	Trichloroacetic acid soln. before addn. and pptn. of rabbit blood	4	8	3.7	6.6
B. & M.	3	25	Sterile, oxalated horse blood laked in distilled water	5	5	3.5	3.6
Micro	3	25	Sterile, oxalated horse blood laked in distilled water	5	5	4.9	4.9

These results indicate that the low recoveries obtained by the Bratton and Marshall method may be due in some cases to incomplete diazotization. The rate of diazotization is influenced by the temperature, sodium chloride and other catalysts, mineral acid content, and possibly by retarding substances in the blood filtrates. If the routine time for diazotization is to be 3 minutes, careful note must be made of these conditions. Higher recoveries (by the Bratton and Marshall method) were frequently obtained when diazotization was carried out for longer than 3 minutes, or when the temperature was markedly higher than 25° C. Addition of sodium chloride to one of the reagents in the regular Bratton and Marshall procedure would preclude the chance of incomplete diazotization, and reduce the time for this step to 1 minute. Cooper, Gross, and Hogan (*3*) recommend diluting the blood with normal saline. Using this method they obtained high recoveries, which they attributed to the prevention of hemolysis on diluting the blood. It might be possible that they are, in part, due to the catalytic effect of the salt on the diazotization.

Table VI. Rates of Diazotization of Sulfathiazole in Various Media

Medium and Method	Per Cent Reaction after Diazotizing for: 0.5 min.	1 min.	2 min.	3 min.	5 min.
			At 25° C.		
Aqueous sulfathiazole solution, B. & M. procedure	46	62	87	90	100
Rabbit blood filtrate, B. & M. procedure	58	79	92	95	100
Aqueous sulfathiazole solution, added NaCl, B. & M. procedure	96	100	100	100	100
Aqueous sulfathiazole solution, micromethod	83	100	100	100	100
Rabbit blood filtrate, micromethod	83	100	100	100	100
Rabbit blood filtrate, micromethod without sulfuric acid	83	96	97	100	100
			At 35° C.		
Aqueous sulfathiazole solution, B. & M. procedure	67	83	100	100	100
Rabbit blood filtrate, B. & M. procedure	83	100	100	100	100
Aqueous sulfathiazole solution, micromethod	100	100	100	100	100
Aqueous sulfathiazole solution, micromethod, without sulfuric acid	93	100	100	100	100

Experimental Procedure

Reagents. Trichloroacetic acid, 3.33 per cent.

Trichloroacetic and sulfuric acid ("acid mixture"). Sulfuric acid (56 ml. of 4 *N*) is added to 1 liter of 3.33 per cent trichloroacetic acid.

Sodium nitrite. An aqueous solution of c. p. sodium nitrite (0.1 per cent) is used. This was found to be very stable (more than a month in summer weather). It was renewed when low readings were obtained from known amounts of sulfa drugs.

Bratton and Marshall's reagent. *N*(1-naphthyl)ethylenediamine dihydrochloride (0.1 per cent in water) was used. It was found possible to use this for a matter of months also, providing the determinations are made against reagent blanks.

Ethyl alcohol, undenatured 95 per cent ethanol.

Instrument. All determinations were made with a Coleman Universal spectrophotometer, with the wave length dial set at 550 mμ. All colors were compared against reagent blanks of the same age, in microcuvettes of 2.5-ml. volume. The similarity in the absorption curves of the dyes formed in the two methods would indicate the method to be equally useful with other colorimetric instruments.

Calibration Curve. Calibration curves were run in distilled water containing the concentration of acids which were present in the regular analyses. All the final colors were formed in 10 ml. flat-bottomed vials. These were found to be convenient, since they do not require racks, and allow complete mixing of the reagents by shaking. To each of a series of vials were added 0.47 ml. of water, 0.43 ml. of 15 per cent trichloroacetic acid, 0.10 ml. of 4 *N* sulfuric acid, and 1.00 ml. of water or one of the various concentrations of sulfathiazole solutions. The procedure gave solutions comparable to 2 ml. of blood filtrate.

Table VII. Rates of Diazotization of Several Sulfa Drugs in Various Media

Medium and Method	Per Cent Diazotized 0.5 min.	1 min.	2 min.	3 min.	5 min.
Aqueous sulfacetamide solution, B. & M. procedure	100	100	100	100	100
Aqueous sulfanilamide solution, B. & M. procedure	46	62	79	92	96
Aqueous sulfapyridine solution, B. & M. procedure	58	75	92	96	100
Aqueous sulfacetamide solution, micromethod	100	100	100	100	100
Aqueous sulfanilamide solution, micromethod	79	98	100	100	100
Aqueous sulfapyridine solution, micromethod	100	100	100	100	100
Sulfathiazole in 2 ml. of distilled water containing CCl_3CO_2H and 1 ml. of alcohol	...	...	...	50	...

The sulfathiazole solutions were made up as follows: 0.1000 gram of c. p. sulfathiazole was dissolved in 1 liter of distilled water. Various amounts of this solution (2, 4, 6, 8, and 10 ml.) were diluted to 100 ml. The resulting dilutions contained 2, 4, 6, 8, and 10 micrograms of sulfathiazole per ml. The color was developed as in the micromethod, using 0.10 ml. of nitrite, waiting 3 minutes, and then adding 1.00 ml. of alcohol and 0.10 ml. of Bratton and Marshall's reagent. By plotting the densities (or the per cent transmission) of the colors against the concentration (2, 4, 6, 8, and 10 micrograms per determination), a very useful calibration curve was obtained. If the outlined procedure for blood is followed, the same scale (2, 4, 6, 8, and 10) will correspond to the milligram per cent of sulfathiazole in the sample taken. In other words, milligram per cent is the same as micrograms per 0.1 ml. Beer's law is exactly obeyed to a concentration of at least 12 micrograms per determination.

Procedure for Blood and Urine

Precipitation of the Blood. Whole blood (0.30 ml.) is added dropwise to 5.70 cc. of trichloroacetic acid, or to the same volume of "acid mixture". Each drop of blood is broken up by vigorous stirring with the end of the pipet, and the tube is agitated by hand after all the blood is added. The mixture is allowed to stand until the precipitated protein settles and is then filtered through either No. 1 or 42 Whatman paper of about 6-cm. diameter. The filtrates are uniformly water-clear. Slightly more than 4 ml. are collected, sufficient for the determination of both "free" and "total" sulfonamides.

Determination of Free Sulfa Drug in Whole Blood. Exactly 2 ml. of the filtrate (from either precipitating acid) are pipetted into 10 ml. flat-bottomed vials, sodium nitrite (0.10 ml. of 0.1 per cent) is added, and at least 3 minutes are allowed for the diazotization. Alcohol (1.00 ml.) is added, and the tube is swirled. On mixing Bratton and Marshall's reagent (0.10 ml.) with the solution, the characteristic color is produced. The formation of the color is complete within 15 seconds, and the densities of the solutions are reasonably constant for 24 hours. Reading in all cases should be made against reagent blanks, and values obtained from a calibration chart.

Determination of Total Sulfa Drugs in Whole Blood. Exactly 2 ml. of the filtrate from the "acid mixture" precipitation, or 2 ml. from the trichloroacetic acid precipitation with 0.10 ml. of 4 *N* sulfuric acid added, are heated in a boiling water bath for about one hour. The volume is adjusted to 2.00 ml. with distilled water, and the procedure for the free determination is followed.

Determination of Sulfa Drugs in Urine. Urine (0.10, 0.20, or 0.30 ml.) is added to the mixed acid blood-precipitating reagent (5.90, 5.80, or 5.70 ml., respectively), representing dilutions of 1 to 60, 1 to 30, or 1 to 20. The pipet is rinsed with the solution. The sample is filtered if any turbidity develops. The solution or filtrate (2.00 ml.) is analyzed according to the method

outlined for blood. The colors formed in the presence of diluted urine are stable for only an hour, with both Bratton and Marshall's and the micromethods.

Summary

A micromethod for the estimation of sulfonamides is based on the coupling of completely diazotized sulfonamides to *N*(1-naphthyl)ethylenediamine. Changes in the procedure have removed most of the sources of objection to the Bratton and Marshall method. The time for a single complete analysis has been reduced to about 8 minutes. The colors formed are stable for 24 hours. Recovery of sulfathiazole added to whole blood has been shown to be essentially complete at a dilution of 1 to 20. On blood from subjects receiving the drug orally, the micromethod gives results which are uniformly about 15 per cent higher than those obtained by the Bratton and Marshall procedure. The micromethod has proved very reliable and useful for large-scale work.

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Microdetermination of Mercury in Organic Compounds

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DESPITE the desirability of a quantitative method for the determination of mercury in biologic products that have been preserved with phenyl mercuric acetate, Merthiolate, or other organic mercury compounds, no entirely satisfactory micromethod has been published. In the author's experience the Gettler and Lehman modification (*2*) of the Winkler method (*6*, *7*) for the determination of mercuric salts, although satisfactory for mercuric ions, has given very low results with organic mercury compounds such as those mentioned, except under certain circumstances when phenyl mercuric acetate may be titrated with dithizone as a monovalent ion. The Gettler and Lehman method consists, briefly, of a nitric acid and permanganate digestion followed by destruction of excess permanganate by nitrite, removal of excess nitrous acid by hydroxylamine sulfate, and titration of the metal ion by dithizone.

Experimental

Since the losses mentioned above might be due either to incomplete digestion or to volatilization, many modifications of the digestion conditions were made in an attempt to avoid these errors. Two methods gave satisfactory results: Carius digestion in a microbomb and treatment of the material with aluminum in a neutral or slightly alkaline medium at 75° to 80° C. The disadvantages of the first were the difficulty in manipulation and the formation of chlorine oxides which had to be removed. The reaction with aluminum provided a practical digestion method and its application to the determination of organic mercurials is the subject of this report. The reaction between metallic aluminum and the organic mercurials studied ran smoothly and quantitatively at 75° to 80° C. at an initial pH range between 7.8 and 8.4.

In order to obtain consistent end points in the dithizone titration, a certain minimum concentration of salts in the aqueous phase is essential. Amounts exceeding the minimum have no detrimental effect. Varying the concentration of acids over the range studied has no effect on the titration (Table I).

Standardization of the titration technique also contributes greatly to the accuracy of the determination, as described below.

Under the conditions described, no evidence of oxidation of the dithizone has been encountered.

Apparatus. Pyrex reflux apparatus consisting of a 50- or 100-ml. round-bottomed flask and Liebig-type condenser with a 25-cm. outside water-cooled jacket.

Reagents. Dithizone Solution. Dissolve 12.5 to 13.0 mg. of diphenylthiocarbazone (Eastman) in 500 ml. of carbon tetrachloride (Baker, analyzed, in bottles), and allow to stand for a day in the dark. It is important to use the best carbon tetrachloride obtainable. Filter through paper and store in a dark brown bottle in the dark.

The titration value of this reagent remains constant for at least a month.

Hydroxylamine Sulfate Solution. Dissolve 20 grams of hydroxylamine sulfate (Eastman) in 100 ml. of water.

Table I. Variations in Titration of Mercury with Dithizone

(0.109 mg. mercury in the presence of different concentrations of acids and salts)

Amounts of Salts and Concentrated Acids in 100 Ml. of Solution	Dithizone in Titrations			Average
	Ml.	*Ml.*	*Ml.*	*Ml.*
500 mg. of Tyrode's salts[a]				
0.5 ml. of H_2SO_4	11.4	11.3	11.3	11.3
0.5 ml. of H_2SO_4 + 1 ml. of HNO_3	11.4	11.4	11.3	11.4
0.5 ml. of H_2SO_4 + 2 ml. of HNO_3	11.2	11.2	11.3	11.2
0.5 ml. of H_2SO_4 + 3 ml. of HNO_3	11.3	11.3	11.4	11.3
0.5 ml. of H_2SO_4 + 5 ml. of HNO_3	11.3	11.2	11.2	11.2
No added salts				
0.5 ml. of H_2SO_4	10.2	9.7	9.9	9.9
0.5 ml. of H_2SO_4 + 1 ml. of HNO_3	9.4	9.9	10.0	9.8
0.5 ml. of H_2SO_4 + 2 ml. of HNO_3	9.7	10.1	9.9	9.9
0.5 ml. of H_2SO_4 + 3 ml. of HNO_3	10.3	10.0	9.7	10.0
0.5 ml. of H_2SO_4 + 5 ml. of HNO_3	9.5	11.2	10.3	10.3
0.5 ml. of H_2SO_4 + 5 ml. of HNO_3				
0.5 gram of NaCl	11.3	11.4	11.3	11.3
1.0 gram of NaCl	11.4	11.3	11.2	11.3
2.0 grams of NaCl	11.2	11.3	11.4	11.3

[a] Composition of Tyrode's salts (*4*): NaCl 8.0, KCl 0.2, $CaCl_2$ 0.2, $MgCl_2$ 0.1, NaH_2PO_4 0.05, $NaHCO_3$ 1.0, and *d*-glucose 1.0 gram.

Mercury Standard Solution. Dissolve 500 mg. of mercury in concentrated nitric acid and dilute to 500 ml. Add 10 ml. of concentrated nitric acid to 10 ml. of this solution and dilute to 1000 ml. This solution contains 0.0100 mg. of mercury per 1 ml.

Concentrated Nitric Acid (Baker, C. P., analyzed, or Grasselli).

Concentrated Sulfuric Acid (Baker, C. P., special).

Aluminum Powder (Merck), best quality.

The actual reagents used are specified, since they are known to be satisfactory. Equivalent products of other manufacture may be substituted.

PROCEDURE. Transfer the sample containing 0.10 to 0.20 mg. of mercury to a round-bottomed flask, and adjust with 0.1 *N* sodium hydroxide and 0.1 *N* hydrochloric acid to pH 7.8 to 8.4, using phenolphthalein as indicator. Concentrate the solution to 2 to 3 ml. on a boiling water bath unless volatile mercury compounds, such as Merthiolate, are present, in which case take an aliquot without attempting to concentrate. Add 100 to 200 mg. of aluminum powder and reflux on a water bath at 75° to 80° C. overnight. An ordinary constant-level water bath may be adjusted to hold this temperature with sufficient accuracy by simply regulating the height of the burner. Creeping of the aluminum along the walls of the flask, which reduces the surface exposed to the solution, may be prevented by adding a few drops of ethyl alcohol.

Cool the flask, add 0.5 ml. of concentrated sulfuric acid through the condenser, and replace on the water bath (75° to 80° C.) until nearly all the aluminum has dissolved. Add 5 ml. of concentrated nitric acid by way of the condenser, wash down the condenser with a few milliliters of water, and continue heating until the solution clarifies. Cool the flask and thoroughly rinse the condenser with water into the flask. Transfer the mixture to a pear-shaped 150-ml. separatory funnel, filtering if necessary, and dilute to 100 ml. Glycerol is a satisfactory lubricant for the glass stopper and stopcock of the separatory funnel. Cool the solution, add 5 ml. of hydroxylamine sulfate solution, shake the mixture, and allow to stand a few minutes. Unless salts are known to be present in the original sample, add 0.5 to 1.0 gram of C. P. sodium chloride and titrate.

In performing the titration, the solution must not be exposed to direct sunlight, because this will promote destruction of dithizone. Add 2 to 3 ml. of dithizone solution to the separatory funnel, stopper the funnel, and shake until the carbon tetrachloride layer is bright orange. Unless the amount of mercury is very small, about 20 shakes are sufficient. The shaking of the separatory funnel should be uniform, counting the forward and backward stroke as one shake. Should the carbon tetrachloride layer remain green, add a known amount of mercury standard, correction for which must, of course, be made in the final calculations. Allow the carbon tetrachloride layer to settle, then draw it off, and discard. Add smaller amounts of dithizone and repeat the operation until the time required for the change from the green to orange color is lengthened to 30 to 40 vigorous shakes. Add 0.1- to 0.2-ml. amounts of the dithizone and repeat until the green color remains on 40 shakes and then changes to orange on an additional 20 shakes. When the addition of 0.1 ml. of dithizone gives a green color which remains after 60 shakes, the titration is finished. A little practice may be necessary to obtain accurately reproducible end points but the technique is easily acquired. Titrations should check at least within 0.2 ml. of dithizone reagent (±0.001 mg. of mercury).

When the end point is reached, draw off the dithizone solution remaining in the separatory funnel and add a known amount of mercury standard—for instance, 10 ml. of the dilute standard equivalent to 0.10 mg. of mercury. Shake the solution and allow it to stand a few minutes, then titrate exactly as above. This last step need be carried out only occasionally to standardize the dithizone solution. The standard is introduced at this step only as a matter of convenience; it may be titrated separately if desired.

A blank determination should be carried out, using all the reagents. When the reagents specified are employed, the blank is rarely greater than 0.1 ml. of dithizone reagent.

CALCULATIONS. The titration usually requires 10 to 15 ml. of the dithizone solution for 0.10 mg. of mercury. The amount of mercury equivalent to 1 ml. of dithizone solution is calculated from the titration of the standard given above and the titration of the sample, corrected for the blank, is multiplied by this figure to give the amount of mercury present in the sample.

RESULTS. To determine the reliability of the method under the conditions described, 2 ml. of a 1 to 6000 solution of either phenyl mercuric acetate or Merthiolate were added to various substances such as Tyrode's salts, powdered egg yolk, and antipneumococcus horse serum and the mixtures were analyzed exactly as described above. Table II shows that the recovery of mercury from these mixtures is quantitative.

TABLE II. RECOVERY OF MERCURY FROM MIXTURES CONTAINING PHENYL MERCURIC ACETATE AND MERTHIOLATE

Additional Substances	Theory	Found	Recovery
	Mg.	*Mg.*	%
Phenyl mercuric acetate equivalent to 0.200 mg. of Hg^{++}			
500 mg. of Tyrode's salts	0.200	0.196	98.0
	0.200	0.195	97.5
	0.200	0.198	99.0
	0.200	0.194	97.0
	0.200	0.194	97.0
	0.200	0.196	98.0
500 mg. of Tyrode's salts and 6 drops of ethyl alcohol	0.200	0.204	102.0
10 ml. of antipneumococcus horse serum, type 1	0.200	0.201	101.5
	0.200	0.203	101.5
1 gram of dried egg yolk (powder)	0.200	0.212	106.0
Merthiolate equivalent to 0.167 mg. of Hg^{++}			
500 mg. of Tyrode's salts	0.167	0.159	95.2
	0.167	0.169	101.2
10 ml. of antipneumococcus horse serum, type 1	0.167	0.169	101.2

Discussion

The recovery experiments demonstrate that the method presented is useful in the presence of considerable amounts of salts and organic material for the determination of mercury in phenyl mercuric acetate and Merthiolate. The fact that these compounds contain mercury in two different types of linkage suggests the possible application of the method in determining mercury in other organic mercury compounds. Preliminary investigation of the reaction between aluminum and organic mercury compounds as a possible step in determining much smaller amounts of organic mercury compounds colorimetrically with dithizone has shown definite promise.

The metals most likely to interfere with the determination are copper, silver, gold, and palladium. Of these only copper may be present in significant amounts in biologic products. Methods exist for the separation of copper from mercury (*6*, *7*, *8*) and prevention of interference of copper (*5*). In actual experience, the blanks have been very low on practically all materials examined. It is advisable, however, to have as a control some of the material before the addition of the preservative.

It is recommended by Laug and Nelson (*3*) that during extraction of mercury with dithizone the acidity of the mixture should not be greater than 1.5 *N*. In the procedure here described, the total amount of acid does not exceed 0.9 *N* at the time of titration.

The author has been unable to find any previous report on the effect of salts on the extraction of the mercury.

The dithizone solution used here is approximately 0.0001 *N* and, theoretically (*1*), two molecules of dithizone are equivalent to one atom of mercury. The theoretical and actual titration values are in good agreement.

Acknowledgment

The author wishes to express his appreciation of the criticisms and suggestions of Mary C. Pangborn.

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Microdetermination of Arsenic in Biological Material

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IN THIS laboratory, many satisfactory arsenic determinations have been made by distilling arsenic trichloride and titrating with iodine according to Bang (*1*). Since the end point of this method is not sufficiently sensitive for minute quantities of arsenic, the method was modified by trapping the arsenic trichloride in dilute nitric acid and determining as molybdenum blue according to Sobotka (*5*), whose method was based on that of Morris and Calvery (*3*). Rodden (*4*) determined arsenic as the trichloride in ferrous and nonferrous alloys by distilling it in a stream of carbon dioxide into water, oxidizing with nitric acid, evaporating and drying at 130° C., and reading the molybdenum blue color photometrically. Hubbard (*2*) adapted the method to biological material.

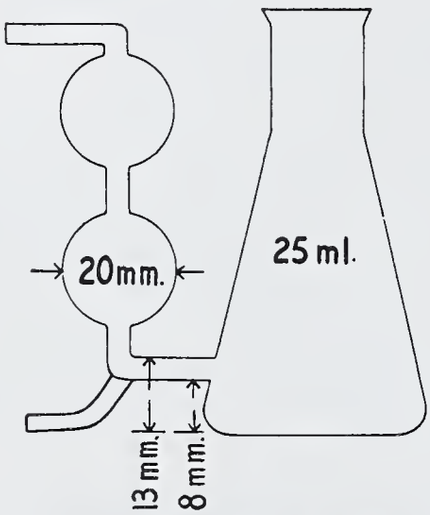

FIGURE 1. FRESENIUS FLASK

In the method described below, arsenic trichloride is distilled rapidly in a stream of hydrogen chloride and trapped in dilute nitric acid.

Apparatus

Kjeldahl flasks, 300 ml., and a Fresenius flask (Figure 1), 25 ml.

Bent connecting tube with rubber stoppers; inside diameter of tube, 4.5 mm. The middle section of the tube slopes slightly toward the receiver.

Erlenmeyer flasks, 25 ml., glass bulbs, electric hot plate, thermostatically controlled (Fisher Scientific Co., Autemp heater), and an electric oven.

Cenco Photelometer (photoelectric colorimeter) with a 625-millimicron (red) filter and 1-cm. cells with a volume of about 8 ml.

Chemicals

Sulfuric acid, specific gravity 1.84, arsenic-free; nitric acid, specific gravity 1.42, arsenic-free.

Potassium chloride, potassium bromide, ferrous ammonium sulfate (Mohr's salt), ammonium molybdate, and hydrazine sulfate (Merck's reagent).

Reagent Solutions

STANDARD ARSENIC SOLUTIONS. Dissolve 1.32 grams of arsenic trioxide (Bureau of Standards) in 30 ml. of *N* sodium hydroxide, dilute, and adjust with *N* hydrochloric acid to pH 6.5 to 6.8. Add sodium bicarbonate to about pH 7.2 and dilute to 1 liter. This solution, containing 1 mg. of arsenic per ml. as sodium arsenite, is stable in that form for many months. Ten milliliters of this stock solution diluted to 1 liter contain 10 micrograms per ml.

COLOR-DEVELOPING SOLUTION. Solution I. Dissolve 1 gram of ammonium molybdate in about 45 ml. of distilled water, add 50 ml. of approximately 10 *N* sulfuric acid, and dilute to 100 ml. This solution is stable for several weeks. Larger crystals of ammonium molybdate appear to yield a lower blank.

Solution II. Take 20 ml. of Solution I, dilute to about 90 ml., and shake. Add 2 ml. of 0.15 per cent hydrazine sulfate solution, dilute to 100 ml., and mix well. This solution is freshly prepared daily.

Procedure

PREPARATION OF THE SAMPLE. The sample or an aliquot is digested with sufficient nitric acid and 5 ml. of sulfuric acid as in the procedure of Bang (*1*) or Morris and Calvery (*3*). Care is taken to maintain an excess of nitric acid until all halogens are removed and all organic matter is oxidized. About 20 ml. of distilled water are added to the cooled digest, which is heated until the sulfuric acid fumes strongly, in order to remove any remaining oxides of nitrogen.

In the decomposition of bone, the oxidation is facilitated by dissolving the sample in reagent fuming nitric acid, specific gravity 1.5, diluting with an equal volume of water, and extracting the fat twice with ether. The residue of the combined extracts is digested separately as above and added to the original nitric acid solution. The calcium sulfate is filtered off and washed well and the digestion of the filtrate is completed as usual. Any loss of the 5 ml. of sulfuric acid is replaced.

DISTILLATION. The Fresenius flask (Figure 1), employed as the receiver and containing 1 ml. of concentrated nitric acid and about 8 ml. of distilled water, is surrounded by an ice-water bath and attached to the connecting tube for distillation. (The amount of distilled water required depends on the construction of the Fresenius flask. It should be sufficient to trap the arsenic trichloride and yet not fill more than two thirds of the lower bulb before bubbles of gas are released when the nitric acid solution is under gradual pressure from the distillation.) The following salts are previously placed in a small covered beaker: 4 grams of potassium chloride, about 0.4 to 0.5 gram of potassium bromide, and 1 gram of ferrous ammonium sulfate. The digest, in 5 ml. of concentrated sulfuric acid in the Kjeldahl flask, is diluted with 6 ml. of distilled water and chilled under the tap. The salts are added all at once through a short wide-stemmed funnel to the flask which is connected immediately for distillation. A strong flame is applied directly to the flask until boiling starts, when it is reduced at once until the tip of the flame just touches the flask. When the effervescence begins to subside and the salts are about half dissolved, the flame is gradually increased until the salts are all dissolved and the solution is boiling rapidly. Meanwhile, the solution in the Fresenius flask is kept under strict observation.

When the first drop of liquid has fallen from the connecting tube into the receiver, the distillation is stopped. The whole time required for this distillation should not be more than 2 to 3 minutes. The receiver is removed and the nitric acid solution is transferred to a 25-ml. Erlenmeyer flask by pouring the solution through the bulbs of the Fresenius flask. The flask is washed twice with 1 ml. of distilled water which is added to the distillate. An aliquot may be taken at this point, especially if the arsenic content is entirely unknown. The nitric acid solution is transferred from the receiver to a 25-ml. glass-stoppered volumetric flask and diluted to the mark, and an aliquot of 10 ml. or less is taken for evaporation. If it becomes necessary to take another aliquot for evaporation, this should be done within 24 hours, because this solution is somewhat unstable after that time. Otherwise, the aliquot should be evaporated with sulfuric acid and redistilled. The size of the aliquot should be such that it contains less than 100 micrograms of arsenic. Spinal fluid and blood containing very small amounts of arsenic should be analyzed directly without taking an aliquot. In this case it is desirable to

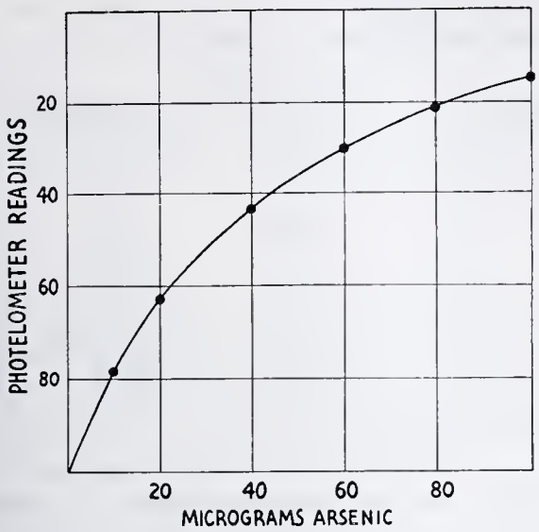

FIGURE 2. CALIBRATION CURVE

use sufficiently pure distilled water to reduce the blank to a minimum.

EVAPORATION AND DRYING. The nitric acid solution in the open Erlenmeyer flask is evaporated almost to dryness as soon as possible and not allowed to stand overnight, because it is easily contaminated with laboratory vapors. This evaporation is done on a thermostatically controlled hot plate. A temperature of 120° to 130° C. is maintained as read on a thermometer suspended in oil in a 30-ml. beaker in the center of the hot plate, so that the bulb of the thermometer touches the bottom of the beaker. (When the evaporating solution has mostly disappeared the temperature may rise suddenly above 130° C. If the temperature is adjusted to 120° to 125° C., it is not apt to rise above 130° at the end of evaporation.) Drying is completed by placing the Erlenmeyer flask in an electric oven at 120° C. for 1 hour. This residue is very stable.

DEVELOPMENT OF COLOR. Exactly 10 ml. of the color-developing reagent solution II are added to the dry residue in the 25-ml. Erlenmeyer flask, which is covered with a glass bulb and heated in a water bath for 10 minutes at 80° to 90° C.

After cooling under the tap, the colored solution is transferred to a 1-cm. cell and compared with water in the comparison cell at a reading of 100 in the photoelectric colorimeter with a 625-millimicron (red) filter. To the reading thus obtained is added a reagent blank correction, which is determined with each set of analyses. From a curve, previously prepared by plotting the corrected Photelometer readings from the distillation of known amounts of arsenic, the amount of arsenic in the sample is determined.

THE REAGENT BLANK. The amount of the blank to be added is determined by placing 5 ml. of concentrated sulfuric acid and 6 ml. of distilled water in a Kjeldahl flask, distilling, evaporating, and reading as in other determinations. The reading from the blank is subtracted from 100 to obtain the blank correction which is added to the readings from known or unknown amounts of arsenic.

Several factors affect the value of the blank. The molybdic acid solution I is stable and will not produce any blank for at least 2 weeks. The nitric acid which is to be employed in the receiver should be stored in a separate reagent bottle, where it is protected from fumes evolved from digestion processes. Distilled water employed in the receiver and in preparing the color developing solutions should be pure and protected from laboratory fumes. Although these fumes do not usually contain arsenic, they increase the color in the blank. When these precautions were observed, a zero blank was obtained. However, since it is sometimes necessary to redistill the water to obtain it sufficiently pure, the blank should be determined until experience has shown that it is unnecessary.

Calibration Curve

It was found more practical to plot the calibration curve as shown in Figure 2 on the basis of the arsenic concentration versus the photoelectric colorimeter readings than against color density values which must be calculated from instrument readings. In practice, two curves are employed, one from 0 to 15 micrograms and the other from 15 to 100 micrograms.

The light filter first employed in the photoelectric colorimeter in this method to measure the absorption of arsenic color reaction had a maximum transmission band at about 625 millimicrons. The bands employed in other methods have been in the range of 567 to 725 millimicrons. Very recently the maximum absorption of the arsenic color reaction was determined as shown by the curve in Figure 3. It is obvious that the maximum absorption is in the infrared at about 840 millimicrons. When a dark infrared gelatin filter was employed in the photoelectric colorimeter, the sensitivity was doubled. A glass filter having the proper wave band is now being obtained. When it arrives, it will be standardized. If it has the maximum transmission at the proper wave band, a new calibration curve will be prepared.

Test of the Method

Arsenic was added to 10-ml. samples of whole blood (Table I) to 50-ml. samples of normal urine (Table II) and to normal mice (Table III). The recovery of arsenic in the three tables is 100 ± 5 per cent except with 1 microgram, where the limits are ±10 per cent. Hence, the accuracy of the method is about 0.2 microgram in the lowest range. No arsenic was found in samples of blood to which none was added. As arsenic is usually found in normal urine, it appears in Table II as a sample blank, although the reagent blank has been reduced to a minimum. In Table III, a sample blank is also found. In order to identify this as arsenic and not phosphorus, which also gives the molybdenum blue color, these tests were repeated and a double distillation was performed in order to remove any

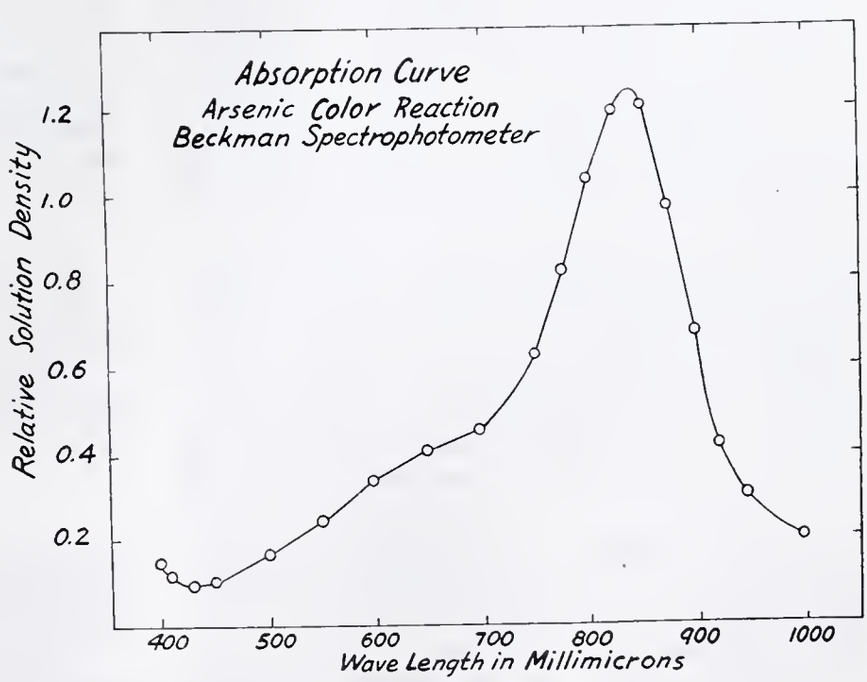

FIGURE 3. ABSORPTION CURVE

Table I. Recovery of Arsenic in Whole Blood

(10-ml. samples)

No.	Arsenic Added	Reagent Blank	Arsenic Found	Arsenic Recovered	
	Micrograms	*Microgram*	*Micrograms*	*Micrograms*	%
1	0.0	0.2	0.2	0.0	...
2	0.0	0.2	0.2	0.0	...
3	0.0	0.2	0.2	0.0	...
4	1.0	0.2	1.2	1.0	100.0
5	1.0	0.3	1.2	0.9	90.0
6	5.0	0.2	5.3	5.1	102.0
7	10.0	0.2	10.0	9.8	98.0
8	15.0	0.3	15.4	15.1	100.7
9	30.0	0.3	28.8	28.5	95.0
10	40.0	0.3	39.8	39.5	99.0
11	60.0	0.3	63.3	63.0	105.0
12	80.0	0.3	78.8	78.5	98.1

phosphorus which might be present in the first distillate. Those mice to which no arsenic was added still gave substantially the same results. Hence, phosphorus was completely eliminated in the first distillation, although it was accumulated from the bones in a relatively large quantity.

Discussion

The apparatus employed in this method, except the photoelectric colorimeter, is of the simplest type. With a good hood there is no necessity for ground-glass joints or other expensive equipment. Since the actual distillation requires only 2 to 3 minutes, only one set of distillation apparatus is required. This consists of nothing more than the Kjeldahl flask employed in the digestion, a bent connecting tube with rubber stoppers at each end, and the small Fresenius flask used as the trap. The time of evaporation of the distillate has been decreased by employing a thermostatically controlled hot plate instead of a steam bath. At 120° to 130° C. there is no loss of arsenic during evaporation. If the temperature of the hot plate is allowed to rise to 130° to 135° C., there is very little if any loss and the time of evaporation is about 1.5 hours. At 140° C. or above the loss of arsenic becomes appreciable. Because of the total savings in time, six to eight samples of blood or urine may be analyzed in an 8-hour day.

No interference was caused by the presence of antimony, bismuth, selenium, or phosphates either in the blank or in the recovery of arsenic. The amount of arsenic in the reagents employed appears to be negligible. In the separation of arsenic by the generation of arsine as in the Gutzeit or Marsh tests, the use of zinc or tin is required. These metals are difficult to obtain entirely arsenic-free in normal times and now the exigency is greatly increased. If the dilute nitric acid employed in the trap and the water employed in the color-developing solution are sufficiently pure and protected from laboratory fumes, the blank may be reduced to zero.

No filtration nor adjustment of the pH value of the color-developing solution is necessary. This solution is placed directly in the dried Erlenmeyer flasks. The characteristic molybdenum blue color produced by heating is stable and obeys Beer's law, as shown when the color density is plotted against the arsenic concentration. The readings in this procedure are made on the transmission through 1-cm. cells. The sensitivity may be increased by placing the same volume of the arsenic solution in longer cells. It is expected also that the sensitivity will be more than doubly increased by the employment of the new absorption filter which is being obtained. Although the range of the color test will be decreased, that is not serious, since a smaller aliquot can be taken. It is planned to continue the investigation of these items and to report in a subsequent paper.

Although this method has been employed successfully in the analysis of about a thousand samples of various biological materials, it is also applicable to any other type of analysis where the microdetermination of arsenic is required.

Table II. Recovery of Arsenic from Urine

(50-ml. samples)

No.	Arsenic Added	Reagent Blank	Arsenic Recovered	
	Micrograms	*Microgram*	*Micrograms*	%
1	0.0	0.0	1.6	...
2	0.0	0.0	1.4	...
3	0.0	0.0	1.4	...
4	1.0	0.0	2.4	90.0
5	1.0	0.0	2.6	110.0
6	1.0	0.0	2.6	110.0
7	5.0	0.0	6.7	104.0
8	5.0	0.0	6.6	102.0
9	10.0	0.0	11.2	97.0
10	15.0	0.1	16.8[a]	101.3
11	20.0	0.0	21.5	100.0
12	40.0	0.1	43.6[a]	105.0
13	60.0	0.1	63.1[a]	102.5
14	80.0	0.0	80.5	98.9

[a] Reagent blank is included.

Table III. Recovery of Arsenic from Normal Mice

No.	Weight	Arsenic Added	Reagent Blank	Arsenic Found	Arsenic Recovered	
	Grams	*Micrograms*	*Microgram*	*Micrograms*	*Micrograms*	%
1	19	0.0	0.4	1.4	1.0	..
2	20	0.0	0.4	1.4	1.0	..
3	18	10.0	0.4	11.0	10.6	96.0
4	17	20.0	0.4	20.9	20.5	97.5
5	18	30.0	0.4	29.9	29.5	95.0
6	18	40.0	0.4	40.7	40.3	98.2

Conclusion

A simple, rapid, and accurate method has been developed for the microdetermination of arsenic in biological materials, including blood, urine, bone, and tissue. The sample is digested with concentrated nitric and sulfuric acids, and the arsenic is distilled as the trichloride and trapped in dilute nitric acid solution in a Fresenius flask. The characteristic molybdenum blue color is developed from the evaporated residue and read in a photoelectric colorimeter with a 625-millimicron filter. The range is 0 to 100 micrograms of arsenic. Based on the recovery of arsenic, the accuracy is about 0.2 microgram in the lowest range. Very recently the maximum absorption of the arsenic color reaction has been found to be in the infrared at about 840 millimicrons. It is expected that the employment of this wave-length band will more than double the sensitivity.

The chief advantages of the method are the simplicity of the apparatus required, the rapidity of the distillation of the arsenic trichloride, and the increased accuracy and sensitivity due to a low reagent blank. This blank may be reduced to zero by the employment of sufficiently pure distilled water.

Acknowledgments

The author wishes to acknowledge his sincere appreciation to Leon A. Sweet and C. Kenneth Banks for their valuable assistance.

He also desires to thank J. M. Vandenbelt for determining the absorption of the molybdenum blue color of the arsenic solution submitted and for drafting the curve shown in Figure 3.

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Mustard Gas in Air

Sensitivity of Qualitative Tests and a Rough Quantitative Determination

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SEVERAL methods for the qualitative detection of β,β'-dichlorodiethyl sulfide (mustard gas) have been described in the literature. Some of these tests were designed for the examination of foods (*6*), and others for the analysis of soils (*4*). Although some papers deal specifically with the detection of mustard gas in air (*1*, *2*), no data are recorded on the minimum concentration of mustard gas in air that can be detected by the several methods. In the event of an air raid in which mustard is used, it would be very desirable to have ready a portable apparatus for the detection of mustard gas, and also to know the sensitivity of the test. A test of sufficient sensitivity would also be useful after the raid to check the effectiveness of the decontamination or the natural disappearance of the poison.

For these reasons several of the more promising qualitative tests were investigated with regard to their sensitivity when applied to contaminated air.

Materials Used

The mustard gas was prepared in this laboratory by William A. Raimond, and the fraction boiling between 115° and 116° C. at 25 mm. was used. It froze sharply at 14.5° C. Comparison of these figures with the recorded literature (*5*) indicates that the compound had a high degree of purity. It was colorless and had only a faint odor of garlic.

The Grignard reagent ($KCuI_2$), alcoholic β-naphthol, and iodoplatinate solution were prepared according to the directions of Stainsby and Taylor (*6*). Filter paper was impregnated with iodoplatinate solution as recommended by Bradley (*1*). A 1 per cent solution of chlorauric acid (*3*) was prepared, and filter paper was impregnated with this solution. A solution of acetic acid, made by diluting 5 ml. of reagent grade acetic acid to 100 ml., and a 0.5 per cent solution of potato starch were prepared. A small quantity of mustard was accurately weighed and dissolved in sufficient glacial acetic acid to give a standard solution containing 0.10 mg. per ml.

Table I. Colorimetric Determination of Mustard Gas Absorbed by Acetic Acid

Test No.	Velocity of Air Streams *A*	*B*	Concentration of Mustard in Mixed Stream	Time	Total Mustard in Mixed Stream	Mustard Absorbed	
	Ml./min.		*γ/ml.*	*Min.*	*γ*	*γ*	%
1	0	1.8	1.0	5.5	10	7.5	75
2	170	1.7	0.010	10.0	17	4	24
3	170	1.7	0.010	5.0	8.5	2	24
4	170	3.4	0.020	10.0	34	10	29
5	170	3.4	0.020	5.0	17	5	29
6	170	3.4	0.020	2.5	8.5	2	24

Apparatus

The apparatus was essentially like that of Yablick, Perrott, and Furman (*7*). A stream of air, denoted as *A*, from a compressor was purified by passage through a mixture of soda-lime and charcoal. The velocity of this stream was controlled by a pinchcock and pressure regulator and was measured by a flowmeter. A second stream of air, *B*, was similarly purified, controlled, and measured and was then bubbled through a vessel containing mustard liquid immersed in a water bath maintained at 20 ± 1° C. The two streams of air were mixed in a T-tube and passed through the test solution contained in a Pyrex test tube, 10 cm. long and 12 mm. in diameter, and fitted with a 2-hole rubber stopper. The delivery tube entered through one hole, extended almost to the bottom, and terminated in a constriction of 1-mm. internal diameter. The other hole held the exit tube which led the air through another mixture of charcoal and soda-lime. The connections between the mustard bubbler and the test tube were glass-to-glass joints covered with tight rubber tubing. The temperature of the laboratory was kept at about 20°.

Table II. Qualitative Tests for Mustard Gas

(Velocity of mixed stream was 170 ml. per minute in all cases.)

Reagent	Concentration of Mustard in Mixed Stream	Time	Total Mustard in Mixed Stream	Results
	γ/ml.	*Min.*	*γ*	
Grignard	0.020	10	34	Negative
$HAuCl_4$	0.040	10	68	Very faint
$HAuCl_4$	0.040	20	136	Faint
β-Naphthol	0.040	10	68	Faint
$HAuCl_4$ paper	0.020	10	34	Negative
Na_2PtI_6 paper	0.020	10	34	Negative

Procedure

The velocity of stream *A* was usually 170 ± 20 ml. per minute, the maximum velocity that could be used without risk of blowing the solution out of the test tube. It also represents a convenient rate for a field test when the air is passed by means of a rubber-bulb aspirator. The velocity of stream *B* was varied between 1.7 and 6.8 ml. per minute in order to vary the concentration of mustard vapor in the mixed air stream.

For the iodoplatinate test, the air was bubbled through 1.0 ml. of 5 per cent acetic acid for the indicated length of time, then the test tube was removed from the apparatus, and one drop of iodoplatinate solution was added, followed by one drop of starch solution. The pink iodoplatinate turns blue if 2 micrograms or more of mustard are present.

The mustard absorbed by the acetic acid can be determined with an accuracy of 1 microgram by comparing the intensity of the blue color with a series of standards. The color standards are prepared by putting 0.02, 0.04, 0.06, 0.08, and 0.10 ml. of the standard mustard solution in Pyrex test tubes (10 cm. by 12 mm.), diluting to 1.0 ml. with water, and adding iodoplatinate and starch solutions. The variation in the concentration of acetic acid does not appreciably affect the intensity of the blue color. The color standards must be prepared at the same time that the color is developed in the unknown solution.

The tests with the Grignard reagent and with chlorauric acid consisted merely in letting the air bubble through 1.0 ml. of the respective solutions and observing at intervals whether a precipitate had been formed. The β-naphthol test was done similarly, except that the test tube was surrounded by a water bath at 40°. The tests with the impregnated papers were performed by putting the papers into the Pyrex test tube and passing the contaminated air stream through this tube. The iodoplatinate paper was moistened with water before use, but the chlorauric acid paper was used dry.

The results of the colorimetric determination of mustard gas absorbed by the dilute acetic acid under various conditions are summarized in Table I. The values in column 4 were calculated from the vapor pressure of mustard (*5*) on the assumption that the air in stream *B* was saturated with the vapor. Most of the entries in column 7 are the mean of duplicate determinations.

The results of the qualitative tests for mustard gas are given in Table II.

Discussion

Test 1 reveals that the absorption of the mustard vapor by the dilute acetic acid is nearly complete when the air velocity is small. Tests 2 to 6 indicate that the absorption is much less complete when the velocity is increased. Nevertheless, mustard vapor can be detected by the iodoplatinate method under the recommended conditions with a 5-minute absorption period when its concentration is only 0.01 microgram per ml. (test 3). Since this concentration requires an exposure of 2.5 hours for fatal results (*3*), the iodoplatinate method is fairly satisfactory for detecting dangerous concentrations of mustard vapor. It is stated (*6*) that this test is not applicable in the presence of oxidizing agents such as chlorine or nitrous fumes or in the presence of reducing agents, including the arsenical vesicants in large concentration.

Table II reveals that the other tests are much less sensitive than the test with iodoplatinate solution.

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Microdetermination of Magnesium with the Polarograph

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A polarographic procedure is given for the determination of microquantities of magnesium as the hydroxyquinolate.

INVESTIGATION of possible chemical changes produced in mouse epidermis by methylcholanthrene (*1*) requires special microprocedures, because of the small amounts of tissue available for analysis. Magnesium, for example, cannot be determined by any ordinary technique, and even polarographic determination is not practical. This metal gives a very poorly defined wave in solutions of tetramethylammonium salts as the supporting electrolyte (*3*, *8*). Müller (*8*) and Heyrovský and Berezicky (*2*) attribute the pronounced maximum, and the ensuing large diffusion current, to the evolution of hydrogen resulting from the rapid decomposition of water at the magnesium-mercury amalgam which is formed. Since 8-hydroxyquinoline has proved to be of great value for the determination of magnesium (*11*), a method for the reduction of magnesium based upon the polarographic reduction of 8-hydroxyquinoline was devised. The author found that this latter compound is reduced at the dropping mercury electrode and that the diffusion current is proportional to the concentration. It was therefore possible to devise a polarographic procedure for the microdetermination of magnesium.

Apparatus and Reagents

Heyrovský polarograph, Model XI (E. H. Sargent & Co.).

A phosphate buffer mixture of pH 7.6, 3.33 *M* with respect to both disodium phosphate and monopotassium phosphate.

A wash solution made by saturating 95 per cent alcohol with magnesium hydroxyquinolate and filtering just before use through a sintered-glass filter covered with a layer of asbestos.

Solution A, prepared by diluting 2 ml. of 1 per cent gelatin (Eastman's de-ashed) solution and 30 ml. of 0.1 *N* hydrochloric acid to 100 ml. with the phosphate buffer solution.

Solution B, prepared by diluting 2.7 ml. of 1 per cent gelatin solution to 100 ml. with the phosphate buffer.

Solutions A and B should be made just before use or stored in a refrigerator, as the presence of gelatin can favor a rapid growth of microorganisms.

Procedure

The investigations of Redman and Bright (*9*) and of Miller and McLennon (*6*) have shown that the quantitative precipitation of magnesium hydroxyquinolate is very critical and much affected by the conditions under which it is carried out. For calibration purposes, the author found that to prevent high results it was necessary to precipitate the magnesium hydroxyquinolate by the method of Kolthoff and Furman (*4*) as follows:

A solution of magnesium chloride (made by dissolving magnesium ribbon in hydrochloric acid) is treated with ammonium chloride and ammonium hydroxide and then heated to 96° to 100° C. To this a 5 per cent alcoholic solution of 8-hydroxyquinoline (Eastman) is slowly added with stirring until a slight excess is present. Digestion is continued for a few minutes and the solution is then filtered hot through a sintered-glass filter. After the precipitate has been washed thoroughly with water followed by alcohol, it is dried at 105° C. overnight.

The calibration data (Table I) were obtained by dissolving 400 mg. of the magnesium hydroxyquinolate in a beaker with 150 ml. of 0.1 *N* hydrochloric acid and transferring this solution quantitatively to a 500-ml. glass-stoppered volumetric flask by rinsing the beaker several times with the phosphate buffer solution. The flask was made to volume with this same buffer solution after the addition of 10 ml. of 1 per cent gelatin. This 2.29 $\times 10^{-3}$ *M* stock solution (nearly saturated) was diluted in 100-ml. glass-stoppered volumetric flasks to give magnesium hydroxyquinolate solutions 1.837 to 0.1148 $\times 10^{-3}$ *M*. Proportionate amounts of 0.1 *N* hydrochloric acid, 1 per cent gelatin, and phosphate buffer were added to maintain a constant pH and equivalent gelatin concentrations. The importance of pH in the reduction of organic compounds has been stressed by Müller (*7*) and by Kolthoff and Lingane (*5*). Solutions in Table I had a pH of 7.09 to 7.12 (glass electrode). Solution A was used for measuring the residual current, i_r.

Table I. Calibration Data for Magnesium in Magnesium Hydroxyquinolate

(Various concentrations of magnesium hydroxyquinolate in a mixture of 7 parts of 3.33 molar phosphate buffer of pH 7.6 and 3 parts of 0.1 N hydrochloric acid at 25° C. Solutions contained 0.02 per cent gelatin. Air removed with nitrogen. h = 64 cm., t = 2.77 sec., m = 2.925 mg. sec.$^{-1}$. Diffusion currents measured at the potentials indicated with respect to the saturated calomel electrode.)

Magnesium Hydroxyquinolate Millimolar	Magnesium equivalent	Residual Current[a]	i_d Observed	i_d Corrected	Potential at Which i_d Was Measured	i_d/C
	Mg./cc.	*Microampere*	*Microamperes*		*Volts*	*Microamp./mmole/l.*
1.14	0.0279	0.39	13.60	13.22	−1.56	11.59
0.918	0.0223	0.31	10.67	10.37	−1.52	11.29
0.860	0.0209	0.31	9.95	9.65	−1.52	11.22
0.5[illegible]0	0.0140	0.29	6.48	6.19	−1.50	10.85
0.459	0.0112	0.27	4.88	4.61	−1.48	10.04
0.2295	0.0056	0.26	2.20	1.94	−1.46	8.44
0.1148	0.0028	0.25	1.07	0.83	−1.40	7.18

[a] Residual current of supporting electrolyte alone.

The diffusion currents were measured (*5*) and the polarograms made after placing the magnesium hydroxyquinolate solutions in shell vials and then removing any oxygen with a stream of nitrogen bubbles. m, the rate of flow of mercury from the capillary in milligrams per second, and t, the drop time in seconds (*5*) were measured in solution A at a potential of −1.5 volts with respect to the saturated calomel electrode. Since the potentials at which i_d were determined decreased slightly with decreasing concentrations of magnesium hydroxyquinolate, the residual currents were measured from −1.56 to −1.40 volts with respect to the saturated calomel electrode. Also i_d/C decreased, but the ratio, except for the 0.2295 and 0.1148 × 10^{-3} M solutions, was found to be fairly constant. For greater certainty in the analysis, a calibration curve was constructed.

Since Maas (cited by Kolthoff and Lingane, *5*) has shown that i_d/C may not be constant when the drop time is too short, the procedure described above was repeated with an electrode having a drop time of 4.38 seconds.

The same general i_d/C relationship was found for this second electrode as with the electrode used for the calibration data in Table I.

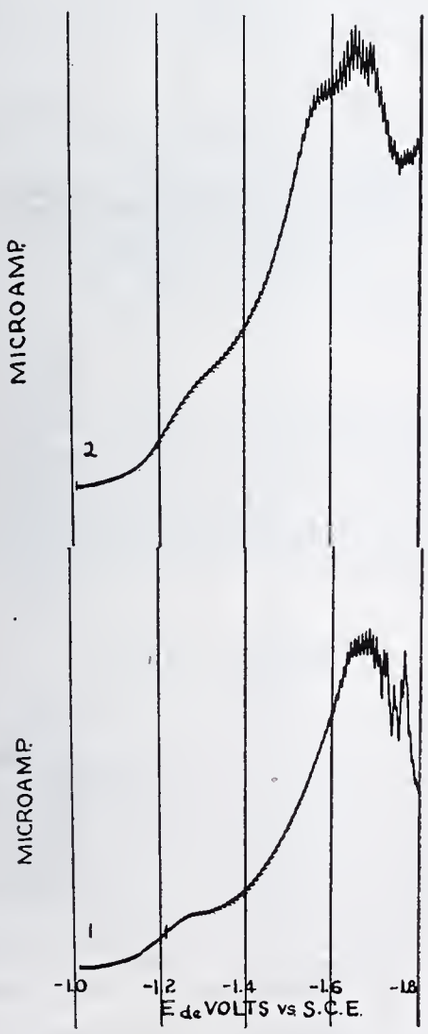

Figure 1. Polarograms of Magnesium Hydroxyquinolate in Phosphate Buffer–Hydrochloric Acid Mixture at 25° C.

1. 2.29 × 10^{-3} M (sensitivity 1/10)
2. 1.337 × 10^{-3} (sensitivity 1/20)

Cathodic Waves of Magnesium Hydroxyquinolate

Solutions 2.29 to 1.72 × 10^{-3} M gave reduction waves which were typical of Figure 1, 1. Two waves, the first very small, were present, but accurate measurement of i_d of the second wave was found to be impractical for analytical purposes because of the small region of constant diffusion current. At a concentration 1.377 × 10^{-3} M, a third wave appeared (Figure 1, 2), the second wave being well defined for 0.914 to 0.1148 × 10^{-3} M solutions and ideal for analytical purposes (Figure 2, 1). Unfortunately i_d of the total double wave (second and third) could not be used because the hydrogen wave interfered with the determination of i_r of the latter (Figure 2, 2). The first small wave (Figure 1, 1) was not present at a concentration 0.2295 × 10^{-3} M.

The half-wave potential of the second wave decreased with decreasing concentrations of the magnesium hydroxyquinolate, the values being about −1.37 and −1.30 volts with respect to the saturated calomel electrode for 0.918 × 10^{-3} M and 0.459 × 10^{-3} M solutions, respectively.

Procedure for Solutions

Solutions of known magnesium content were made by dissolving Baker's magnesium ribbon (acid-washed to remove any oxide) in hydrochloric acid. All solutions were diluted when necessary to a volume of approximately 10 ml. in 50-ml. Pyrex beakers. Various amounts were treated as follows for precipitation of the hydroxyquinolate: To each sample were added 1 ml. of 2 N ammonium chloride and 0.5 ml. of 6 N ammonium hydroxide (method of Kolthoff and Furman, *4*). The solutions were brought to the boiling point, and 0.7 ml. of a 1 per cent solution of 8-hydroxyquinoline in 95 per cent alcohol was added dropwise with stirring. The solutions were allowed to cool, then reheated, and another 0.7-ml. portion was added at or near the boiling point in the same way. This procedure was repeated for a third treatment. Another group of standard solutions was treated with 2-ml. portions of the 8-hydroxyquinoline at room temperature after the addition of ammonium chloride and ammonium hydroxide, and these solutions were also held at or near the boiling point for 10 minutes.

After one hour's standing at room temperature, the precipitates were aspirated from the mother liquor with 10-mm. disk Pyrex filter sticks (medium porosity) covered with a layer of asbestos. The precipitates were washed with two 2-ml. portions of wash alcohol which had been previously filtered as described above, and precipitates, filter sticks, and beakers were dried at 105° C. for 0.5 hour. After cooling to room temperature, 3 parts of 0.1 N hydrochloric acid were added, and the difficultly soluble hydroxyquinolate was brought into solution by gentle warming and stirring. While still warm, the sides of the beaker were rinsed with the acid, using the filter stick to dislodge any particles of hydroxy-

quinolate adhering to the walls. Finally, the beaker was tilted so that the lower third of the filter stick was rinsed by the warm acid, 7 parts of solution B were added, and, after thorough mixing, the final solution was filtered through paper to remove the asbestos. A sufficient quantity of acid and of solution B was used to keep the magnesium hydroxyquinolate at or below 1×10^{-3} *M*.

Since the half-wave potential decreased with decreasing concentration of the magnesium hydroxyquinolate (Table I), the potential at which the diffusion current was measured was determined as follows: When the diffusion current was reached (shown by the region of constant deflection of the galvanometer oscillations), the voltage applied to the dropping mercury electrode was noted. Then as soon as the galvanometer oscillations indicated that the region of constant diffusion current was passed, the applied potential was again observed. The mean of the initial and final applied potentials was the potential at which the diffusion current was measured, and also the potential from which the residual current was taken from Table I. The magnesium content was read from the calibration curve.

Typical recoveries from various samples of known magnesium concentration are shown in Table II. Quantities as small as 68 to 100 micrograms could be determined with an error of ±1.8 to ±2.8 per cent. Amounts of magnesium up to 0.563 mg. were determined equally well by adding the 8-hydroxyquinoline to a hot or cold solution of the known, provided ammonium chloride and ammonium hydroxide had been added previously. Since 2.8 micrograms (0.1148×10^{-3} *M* magnesium hydroxyquinolate) per ml. had a diffusion current of 0.825 microampere (corrected), the method might serve for the determination of mere traces of magnesium with adequate methods for handling minute amounts of precipitate. This method should also be of value for the microdetermination of the other metal ions which are quantitatively precipitated by 8-hydroxyquinoline (*11*).

Table II. Assay of Known Magnesium Chloride Solutions

Magnesium Added	Recovered	Difference
Mg.	*Mg.*	*Mg.*
0.5625	0.5570	−0.0055
0.3369	0.3400	+0.0031
0.2250	0.2225	−0.0025
0.1800	0.1750	−0.0050
0.1350	0.1370	+0.0020
0.1125	0.1140	+0.0015
0.1000	0.1020	+0.0020
0.0800	0.0815	+0.0015
0.0680	0.0700	+0.0020

Procedure for Tissues

The following technique has been used for the determination of magnesium in 100 to 400 mg. (wet weight) of mouse epidermis:

The mouse epidermis was ignited at 450° C. in a silica crucible in a muffle furnace until all the organic matter was destroyed. The ash remaining was dissolved with 1 ml. of 0.1 *N* hydrochloric acid, and the crucible rinsed into a 50-ml. Pyrex beaker with four 2-ml. portions of distilled water. (Since the calcium content of mouse epidermis had been previously found to be low, *10*, and the iron content very low, *1*, removal of these ions was unnecessary. Iron and calcium are the only elements found in sufficient quantity in limited samples of animal tissue that might interfere with the precipitation of magnesium hydroxyquinolate as described below.)

The magnesium was then precipitated by adding the 1 per cent alcoholic 8-hydroxyquinoline reagent to the sample at 96° to 100° C. as previously described. The precipitate was washed, dried, and brought into solution as outlined above.

From the diffusion current, the magnesium content could be read on the calibration curve. When working with unknowns it was found convenient to have solution A at hand, so that an aliquot of this mixture could be added to one of the buffered magnesium hydroxyquinolate without changing the final pH. This addition became necessary at times in order to keep the molarity at or below 1×10^{-3}. However, after a few trials, it was not difficult to estimate the proportions of 0.1 *N* hydrochloric acid and of solution B required to keep the diffusion current within the range of the calibration curve.

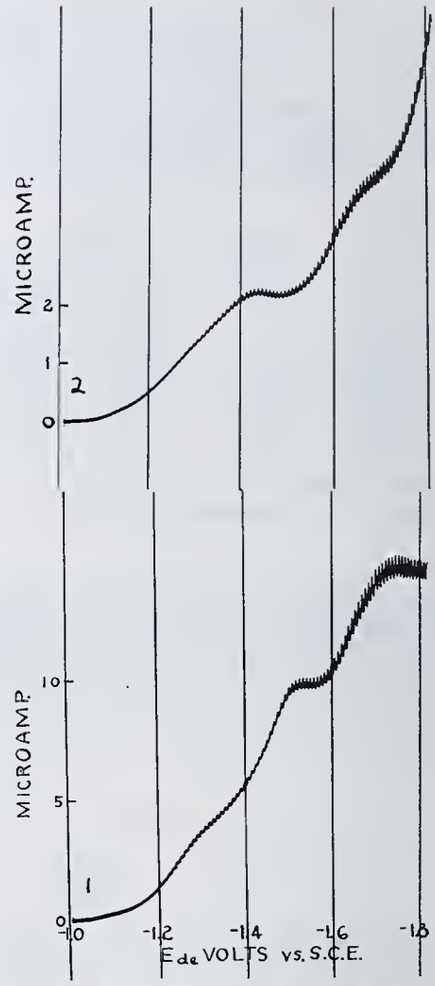

Figure 2. Polarograms of Magnesium Hydroxyquinolate in Phosphate Buffer–Hydrochloric Acid Mixture at 25° C.

1. 0.86×10^{-3} *M*
2. 0.2295×10^{-3} *M*

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Reproducibility of Weighings Made on Microchemical Balances

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AT THE Detroit Meeting of the AMERICAN CHEMICAL SOCIETY in September, 1940, G. E. F. Lundell, chairman of the newly organized Division of Analytical and Micro Chemistry, asked the divisional secretary to form a Committee on Microchemical Balances, for the purpose of formulating specifications for performance and suggestions for materials and construction of these instruments for the benefit of American balance manufacturers.

At the meeting of this committee at Atlantic City in September, 1941, it was decided first to find out what performance, as regards reproducibility of weighing, was actually being obtained by microanalysts in the course of their regular work. To obtain this information, the members of the division were circularized with the request that they perform a test under definitely stated conditions and report their results to the committee secretary. These have been summarized and the necessary statistical calculations performed by the divisional secretary, Rev. Francis W. Power, S.J., Fordham University, and were presented in substantially this form at the Buffalo Meeting in September 1942.

TABLE I. SUMMARY OF REPRODUCIBILITY EXPERIMENTS ON MICROBALANCES

(Requested by the Microbalance Committee of the Division of Analytical and Micro Chemistry, 1942)

Type and Serial No.[a]	Date: Purchase	Date: Recondi-tioning	Standard Deviation: 1-gram load	Standard Deviation: 10-gram load
			Micrograms	
K 2741	1935	....	7.4	7.2
K 2531	1935	1937	2.8	...
K 2776	1936	....	3.2	...
B (special)	1941	....	12.8[b]	13.0[b]
K 2340	1930	1936	6.6	6.3
K 2943	1938	....	3.1	4.9
			2.8 (recheck)	3.9
K 2731	1936	....	3.6	5.2
K 2413	1931	....	6.1	8.3
K 2447	1933	....	1.0	1.6
B 27,225	1937	1939	22.3[b]	17.0[b]
K 2561	1933	1939	14.0[b]	38.0[b]
B none	1935	....	5.9	3.8
K 2769	1937	....	1.7	2.1
A 14,772	1942	....	1.2	2.1
K 2881	1937	....	2.2	2.8
A (special)	1936	....	0.9	1.1
B 31,636	1942	....	9.9[b]	28.3[b]
			8.4[b] (recheck)	22.2[b]
			5.1[c]	5.2
K (not given)	1937	....	2.5	6.4
B 29,734	1940	....	1.2	0.7
K 2863	1937	1940	1.8	2.3
K 2790	1937	1940	4.7	6.7
K 2423	1933	1939	2.2	1.7
B 26,730	1939	....	3.3	2.5
B 30,833	1941	....	4.0	5.2
K 2877	1938	....	5.9	5.6
K 2545	1934	....	2.2	3.6
B 21,406	1930	1935	8.0	6.5
S-R 6164	1938	....	3.5	2.0
A 12,745	1940	....	1.3	1.3
Results on Other Balances				
A semimicro B X 11,661	1941		4	8
B macro 22,984	1931		21 (2-gram load)	27 (50-gram load)
S macro 20,450	1915		52 (20-gram load)	.....
B semimicro 24,675	1933		6 (5-gram load, Sept., 1938)	.....
B semimicro 24,675	1933		16 (5-gram load, Oct., 1942)	.....

[a] Designations of balances: K, Kuhlmann; B, Becker; A, Ainsworth; S-R, Sartorius (Ramberg).
[b] Not used in establishing median.
[c] After elimination of vibration.

TABLE II. CONDENSED SUMMARY OF RESULTS (OCTOBER, 1942)

Number of microbalances studied	29
Standard deviation of individual weighings (median value)	±3.4 micrograms
Probable error of individual weighings	±2.3 micrograms
Probable error of individual weighings, Corner and Hunter	±3.2 micrograms
Largest error to be expected in any one weighing (twice standard deviation)	±7 micrograms

The test consisted in weighing two 1-gram weights one against the other, each weighing being followed by a zero point determination. The rider was required to be reset for each weighing and for each zero point. Details of reading the scale, proper conditions of temperature equilibrium, minimizing vibration effects, etc., were left to the discretion of each analyst, who was asked simply to perform these weighings with the usual careful technique he would use in a regular series of micro weighings such as those involved in a succession of carbon and hydrogen determinations. The cooperators were asked to make a series of ten such weighings on the 1-gram weights and another series of ten using two 10-gram weights; the number ten for the series was selected as a compromise between the requirements of statistical stability for the standard deviations and the amount of time that a busy analyst could be reasonably expected to spend on such a task.

The standard deviations which are given in Table I are those of the individual weighings, and were calculated from the formula

$$s = \sqrt{\frac{\Sigma d^2}{n - 1.5}}$$

where d is the deviation in micrograms of each of the $n(=10)$ weighings from the mean. The number in the denominator is decreased by 1.5 to correct for the underestimation of a standard deviation derived from such a short series. Further calculation indicates that there is no significant difference between the values for 1-gram load and those for 10-gram load.

In the summary, Table II, the median values for the standard deviations are also expressed as "probable errors" in order to make the data more easily comparable with a somewhat similar study made by Corner and Hunter (*4*) which came to our notice about the time that our requests for data were being sent out. They give figures for the probable error of weighings of two 1-gram weights made on 8 different microbalances, one of which (an Oertling) was studied extensivery. The standard deviations in their paper vary from 2.5 to 12.6 micrograms, the poorer values being obtained on rather old balances used by students. On the whole their study leads to a probable error for the individual weighings of about ±3 micrograms, which is in very good agreement with the results of the present survey. They also conclude that most of this error is due to the setting of the rider.

Our figures, as well as those of Corner and Hunter, obviously upset any chemist's pious belief that because his

microchemical balance will respond to a difference in weight of 1 microgram it will also reproduce weight differences as closely as this on repeated trials. If we assume that the outside tolerance or maximum error to be expected is twice the standard deviation, we find that by and large our analysts should work on about 7-mg. samples if they wish to be reasonably sure of their sample weights to 1 part per 1000, the value to be used by any given analyst depending on the reproducibility figure which he gets. The actual sample weight for the over-all process is also controlled by its chemical factor and of course by the error that one is willing to tolerate for the whole analysis. This matter has been fully discussed by Benedetti-Pichler (*1*, *3*), who gives figures for the reproducibility of weight by some analytical balances. The discussion in his book should also be read in this connection (*2*). Seeing that only about one third of the reported figures are such as would permit 5-mg. samples to be weighed to within 1 part in 1000, it is recommended that the analyst check his balance by some such reproducibility test as described here, and be guided by what he may find.

Conclusion

While we do not as yet wish to go on record as recommending a certain maximum reproducibility as a specification, we feel that the foregoing figures warrant an expression of surprise at the poor performance of so many microchemical balances (including some domestic ones) and a recommendation to the analyst to ascertain for himself just how well his own balance will actually perform.

The work of the committee along lines of performance specifications and suggestions for materials and construction is being continued, and the chairman would appreciate suggestions from those interested. We wish also to express our gratitude to all those who have cooperated in making these tests:

Cooperators	Cooperators
Rieman (Rutgers)	Rodden (N. Bur. Standards)
Kuck (C. C. N. Y.)	Chapman (Cyanamid)
Nichols (Cornell)	Haagen-Smit (Cal. Tech.)
Tuemmler (Shell Development Co.)	Bushey (Aluminum Co.)
Power (Fordham)	Hallett (Eastman Kodak)
Alicino (Fordham)	Duncan (W. Va. University)
Royer (Calco)	Galitzine (G. E., Pittsfield)
Shrader (Dow)	Clarke (Wesleyan)
Van Etten (Northern Regional Lab.)	Signeur (Canisius)
Huffman (Denver)	

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Detection of Gold in Plating

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TESTS to detect gold in platings must frequently be made in U. S. Customs Laboratories, as the Tariff Act (*3*) prescribes different rates of duty for plated and unplated articles. Various methods have been proposed for the electrographic detection of gold without material injury to the surface. Each is limited in either failing to reveal gold in very thin "flash" plates or in not providing a sharp distinction between gold and other metals.

Arnold (*1*) uses a solution of benzidine in acetic acid for the electrolysis, but differentiation between gold and copper in flash plates on brass is extremely difficult, as both produce similar greenish-blue colorations. Calamari, Hubata, and Roth (*2*) use a peroxide solution containing nitrate ion, but their method, as admitted by the authors, is inapplicable to thin gold plates on copper or brass because the characteristic gold coloration is masked by a strong greenish brown from the copper. Yagoda (*4*) electrolyzes with a 1 per cent sodium chloride solution and follows with a spot test using stannous chloride, but results are negative with very thin gold plates.

For the past 6 months, this laboratory has used the following method, which yields a distinctive gold test even for thin plates without interference from other metals or alloys yet encountered.

Apparatus and Test Solution

Three No. 6 dry cells, connected in series, are used (approximately 4.5 volts). A small clamp is connected to the anode, a piece of platinum wire (B. & S. gage 18) to the cathode. The test solution is prepared by adding approximately 10 grams of stannous chloride to 100 ml. of dilute sulfuric acid (1 to 9). Any precipitate formed on mixing can be ignored. A piece of metallic tin should be added to the solution to prevent its oxidation.

Procedure

The anode clamp is attached to the article to be tested after the removal of any lacquer present thereon. A piece of spot-test paper or absorbent filter paper moistened with a drop of the test solution is then placed against the spot on the metal surface to be tested. The platinum cathode is used to press the moist paper against the metal surface for a period of about a second. If any doubt arises as to whether or not a closed circuit has been obtained, a milliammeter may be inserted in the circuit. An intense purplish-brown stain on the side of the paper in contact with the specimen indicates the presence of gold.

If silver is present in the object under examination, the test should not be performed in direct sunlight, nor be exposed too long to diffused light, as the purple color of the partially reduced silver chloride will develop in a short time.

Interferences

Approximately forty common metals and alloys have been tested by the above method and negative results obtained in each case.

Nickel, cadmium, zinc, tungsten-nickel-tin alloy, brass, copper-vanadium alloy, titanium, silver, phosphor-tin alloy, copper-molybdenum alloy, sterling silver, tungsten-nickel alloy, copper, molybdenum, tungsten, iron, carbon steels, stainless steels, tungsten-tin-copper alloy, chrome-titanium alloy, nickel-silicon alloy, platinum, rhodium, tin-nickel alloy, lead, pewter, tin solder, Monel metal, bronze, German silver, antimonial lead, type metal, tantalum, Babbitt metal, aluminum, bismuth-lead alloy, magnesium, tin, and indium gave no perceptible colorations.

Chromium gave a light yellow and cobalt a very light pink color.

Note. While extensive tests have not been made on gold alloys, this method gives excellent results with the usual 10-, 14-, and 18-carat jewelry.

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INDUSTRIAL AND ENGINEERING CHEMISTRY

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A Vacuum Sublimation and Molecular Distillation Apparatus

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A molecular distillation and vacuum sublimation apparatus, for laboratory use, consists of a manifold maintained at 10^{-5} mm. and an auxiliary degassing line. The manifold is equipped with twelve ground-glass openings of various sizes for use with different types and sizes of stills. A description of some of the stills is included.

INVESTIGATIONS with compounds of high molecular weight, such as steroids and carcenogenic hydrocarbons, led to the construction of a molecular distillation and vacuum sublimation apparatus for purposes of purification and separation. To minimize the time necessary to accomplish these operations, an apparatus was desired which would embody the following features: (1) immediate availability of a high vacuum to a number of operators; (2) distillation and sublimation apparatus useful for macro to micro quantities; (3) arrangement of the apparatus so that the distillations or sublimations could be started or stopped at will; (4) preservation of products in case of mechanical failure of the system; (5) continuous operation of the system with a minimum of attention; and (6) electrically heated stills that would require little or no attention. To fulfill these needs the apparatus shown in Figures 1 and 2 was constructed.

This apparatus consists primarily of two vacuum lines, the manifold which is maintained at a free air pressure of 10^{-5} mm. (McLeod gage), and the exhaust line which is maintained at 10^{-3} mm. The exhaust line serves not only to complete the degassing of the distilland, but also to effect partial evacuation of the still which then may be connected to the manifold without allowing an appreciable rise in pressure. The introduction of a new distillation unit does not, therefore, impair the efficiency of the distillations already in progress. Twelve standard-taper ground-glass outlets are placed along the manifold at intervals of 20 cm. Between the manifold and each outlet are placed wide-bore stopcocks (Eck and Krebs No. 5064), whereas stopcocks of narrower bore (Eck and Krebs No. 5096) connect each outlet with the exhaust line. These are arranged as shown in Figures 3 and 4.

Stopcocks of varying sizes are used. The necessity for this arises from the variation in size of the ground-glass outlets which, in turn, is determined by the design and size of the stills employed. The two largest outlets (size 24/40, stopcock 10 mm.) occupy the center positions on the manifold and thus are nearest the source of vacuum. The two outlets on either side of these are somewhat smaller (size 19/38, stopcock 8 mm.), whereas the smallest outlets (size 14/35, stopcock 6 mm.) occupy the three positions at each end (Figure 2). The stopcocks between the outlet and exhaust are all of the same size (4 mm.). Each outlet is provided with a ground-glass plug which serves to prevent exposure to the atmosphere and dust when not in use.

For construction of the manifold, 40-mm. Pyrex tubing is employed, whereas the exhaust line is constructed of 10-mm. tubing. The assembly of the apparatus is facilitated by constructing it in two halves, which are connected after being mounted on the rack. In this way the possibility of strain is diminished.

The low pressure in the manifold is maintained by an all-steel mercury diffusion pump (Cenco Supervac) and a motor-driven backing pump (Cenco Pressovac). A dry ice trap between the pumps and the manifold prevents condensable gases from entering the pumps and mercury vapors from entering the manifold. A stopcock (Eck and Krebs No. 5044, 15 mm.) between the mercury diffusion pump and the backing pump allows maintenance of the vacuum, even though the pumps are stopped. Para-rubber tape (Cenco) has proved effective for metal-to-glass seals on the pumps and stopcock.

At one end of the manifold is a McLeod gage, protected by a dry ice trap. This gage has been employed for convenience, and does not represent the real pressure of residual vapors in the distillation units. It does, however, record air leakage accurately and give a valuable indication of vacuum-tightness. The true pressure of residual vapors could be measured by the use of a Pirani gage attached to the still with a suitable ground-glass joint.

An ordinary rotary pump (Cenco Hyvac) has proved adequate for the maintenance of the low pressure in the exhaust line. This line is likewise provided with a McLeod gage for which a trap is unnecessary, since the pressure reading is of secondary importance, serving only to indicate when the substance to be distilled has been degassed and the still evacuated.

The rack upon which the apparatus is mounted is constructed 3.75 × 3.75 × 0.3 cm. (1.5 × 1.5 × 0.125 inch) channel iron welded at the corners. The uprights, upon which the manifold, exhaust line, heaters, stills, McLeod gages, and traps are clamped, are 1.25-cm. (0.5-inch) rods mounted 20 cm. apart and bolted to the main frame. In order that the traps may be accessible, no upright is placed in the center (Figures 1 and 2). The rack is mounted 28 cm. from the wall in order to furnish room for a shelf upon which the pumps are placed.

Heaters

To provide a constant and easily adjustable source of heat, without use of an oil or metal bath, electrically heated air baths were constructed. These consist primarily of an asbestos-covered can and lid provided with a Nichrome heating element and a supporting clamp. The dimensions of each heater are determined by the size of the still for which it is to be used. The heating element consists of Nichrome resistance wire (No. 28),

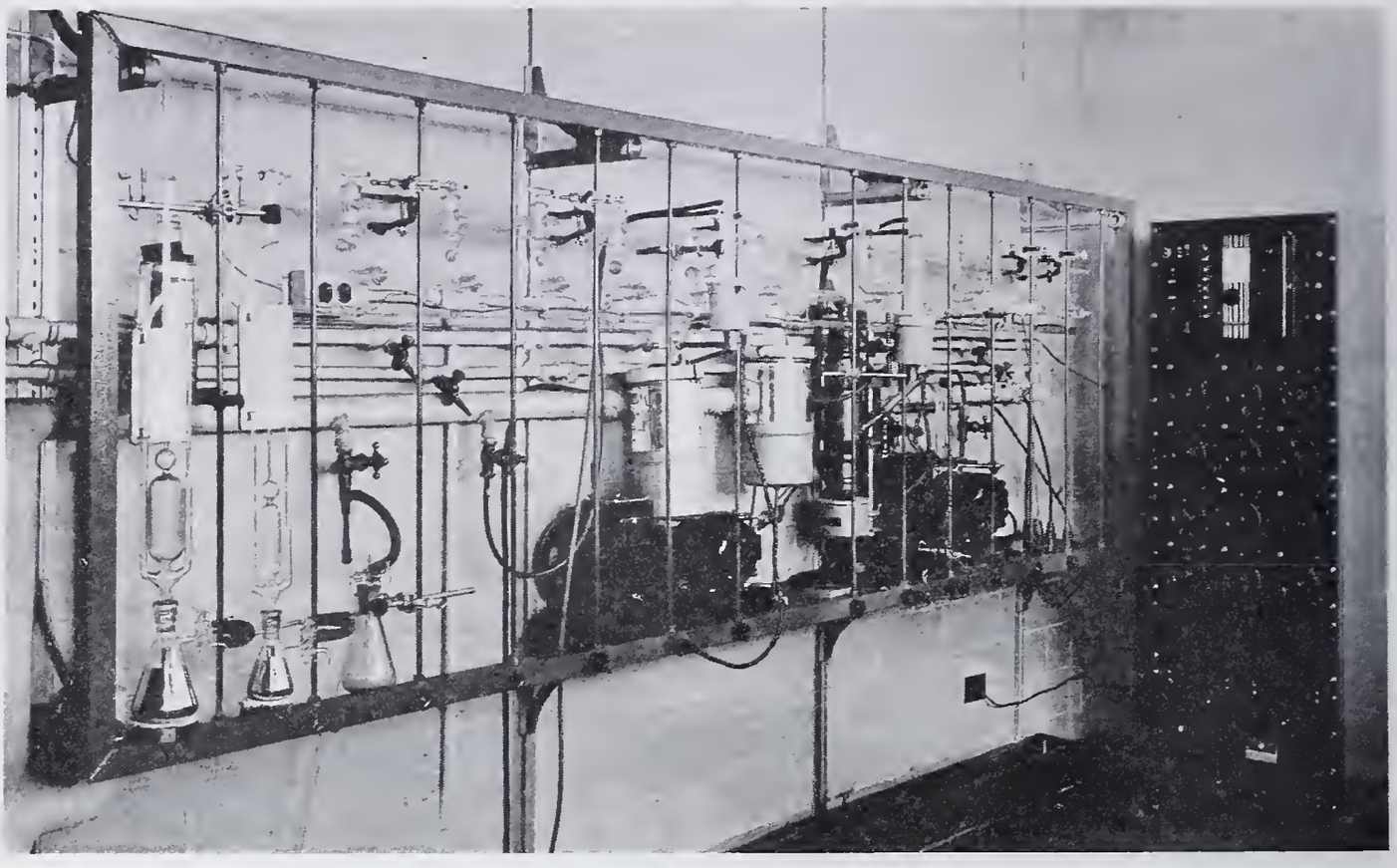

FIGURE 1. MOLECULAR DISTILLATION AND VACUUM SUBLIMATION APPARATUS

one half of which is coiled. A length is chosen (3 meters) which gives about 300 watts at 110 volts. The coiled end of the wire is fastened at *A* (Figure 5) and the coil is distributed over the bottom of the can. The remainder of the wire is threaded through a hole at *B*. Portion *C* is insulated with asbestos, after which the remainder of the wire is coiled around the outside of the can. The outside coils are covered with asbestos, whereas the inner coils are covered with a thin layer of alundum cement. A porcelain washer at *B* serves to insulate the wire. The terminals at *A* and *D* consist of a brass bolt and several brass nuts insulated with mica washers. Porcelain insulators could also have been used. After the asbestos has dried it is coated with water glass to retard abrasion.

The heater support consists of two parts: a brass bracket, *E*, riveted to the can, and a brass rod, *F*, with a vertical hole drilled so that *E* fitted tightly inside it. A brass or iron rod, *G*, is fastened securely to *F* with a set screw. The heaters are supported by clamping *G* to the uprights with standard clamp holders. Figure 4 shows a still and heater in operation.

Temperature Control and Measurement

The temperature adjustment of the distillations is accomplished by the use of autotransformers (General Radio Co., Variac, Type 200-CU). These, provided with switches and pilot lights, are mounted on a panel board placed near the distillation apparatus. A complete wiring diagram is shown in Figure 6. Fuses (5 amperes) protect the Variacs from any overload resulting from failure of the heaters. The leads from the panel board to the heater outlets are placed in the channel of the bottom crosspiece of the main rack. The outlets, which are standard lamp type, are bolted to the frame, as shown in Figure 2. The heaters provided with suitable plug and cord can be used interchangeably at any of the twelve outlets.

A Leeds & Northrup potentiometer type temperature indicator (No. 8674-BC) assures temperature measurements accurate to 2° C. within a range of 0° to 400° C. Each heater cover is provided with an opening into which may be inserted an iron-constantan thermocouple encased in a glass tube.

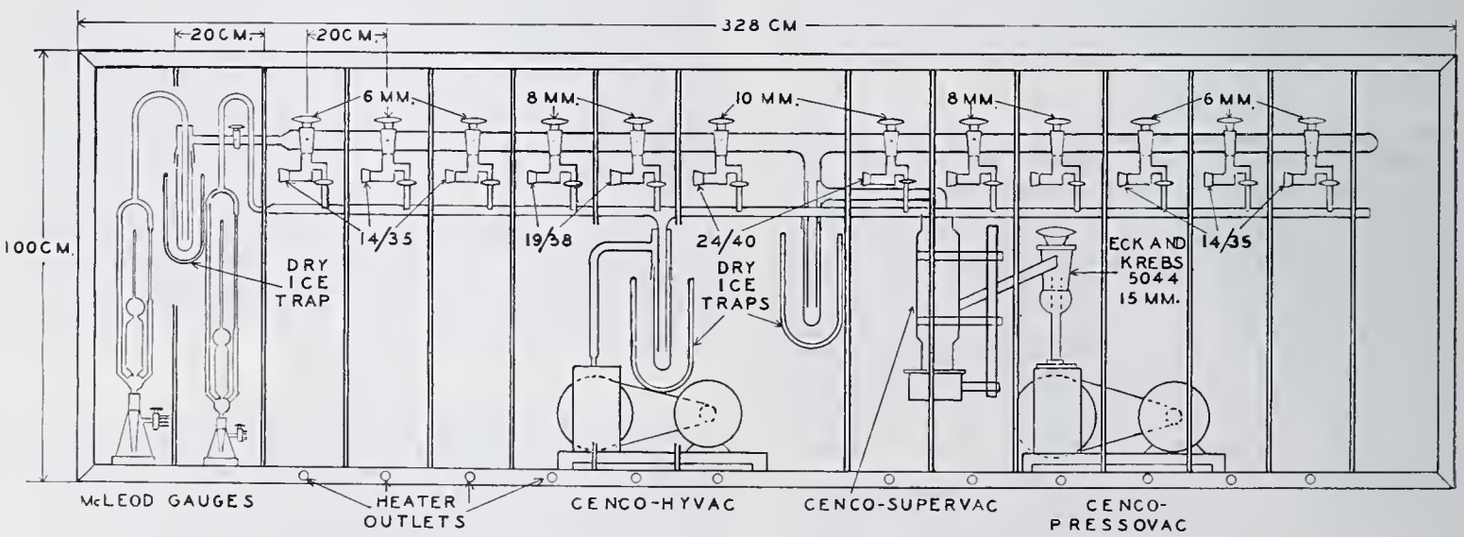

FIGURE 2. DIAGRAM OF APPARATUS

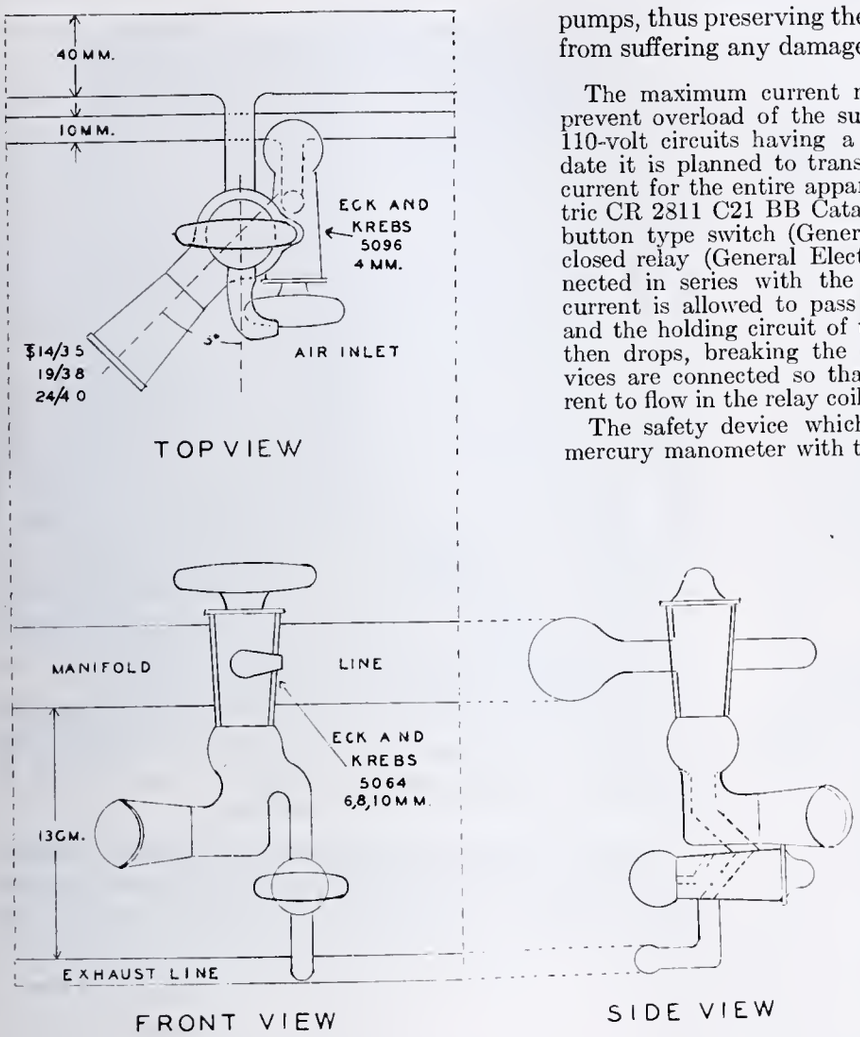

FIGURE 3. ARRANGEMENT OF STOPCOCKS AND OUTLETS

The temperature indicator is placed on the panel with the Variacs. The thermocouple leads, from the panel to the heaters, are fastened along the length of the exhaust line.

Safety Devices

Since many of the distillations and sublimations are allowed to run a day or more without attention, it seemed advisable to provide for the preservation of the products in case of failure of the system. Operation of the system could be interrupted in two ways: (1) distillation of the mercury from the diffusion pump into the backing pump and into the trap, owing to an inadequate water supply in the condenser; (2) breaking of the vacuum by mechanical failure of the backing pump, by development of cracks in the glass system or by sudden leakage of one of the stopcocks.

The safety devices were arranged so that they would interrupt the current to the heaters and to the vacuum pumps, thus preserving the products being distilled and preventing the pumps from suffering any damage.

The maximum current required for the entire apparatus is 45 amperes. To prevent overload of the supply circuit, this total load is divided between three 110-volt circuits having a maximum capacity of 15 amperes each. At a later date it is planned to transfer this load to a 220-volt three-phase circuit. The current for the entire apparatus passes through a magnetic switch (General Electric CR 2811 C21 BB Catalog No. 6,938,875 BB2) which is actuated by a push-button type switch (General Electric CR 2943-A200A start-stop). A normally closed relay (General Electric CR 2811-C9A Catalog No. 4,980,696 G2) is connected in series with the holding current of the magnetic switch. When a current is allowed to pass through the relay coil, the load circuit of the relay and the holding circuit of the magnetic switch are broken; the magnetic switch then drops, breaking the current to the heaters and pumps. The safety devices are connected so that in case of failure of the system they allow a current to flow in the relay coil.

The safety device which operates in case of vacuum failure consists of a mercury manometer with two sealed-in leads. This is placed beside the McLeod gage, so that the dry ice trap will prevent mercury vapor from entering the manifold.

The safety device which operates in case of failure of the water through the condenser of the mercury diffusion pump consists of a mercury-filled flowmeter type of apparatus. The leads are arranged so that they are not connected as long as sufficient water flows. Should this not be the case, however, the two leads are connected by the mercury, thus closing the circuit, which again allows the current to flow through the relay coil. A simple and convenient device of this type was designed by Romeo W. Gouley (*3*).

A switch is provided to break the safety circuit whenever it is desired to start the system. A pilot light indicates whether or not the safety devices are in operation. The magnetic switch, the push-button switch, the relay, the safety switch, and pilot lights are mounted on the panel board (Figure 1).

Types of Stills

The flexibility of the apparatus allows the use of various types and sizes of stills and sublimation apparatus. Figure 7 shows an improved type of still which uses a cold-finger condenser inserted into the distilling flask by means of a ₮ joint.

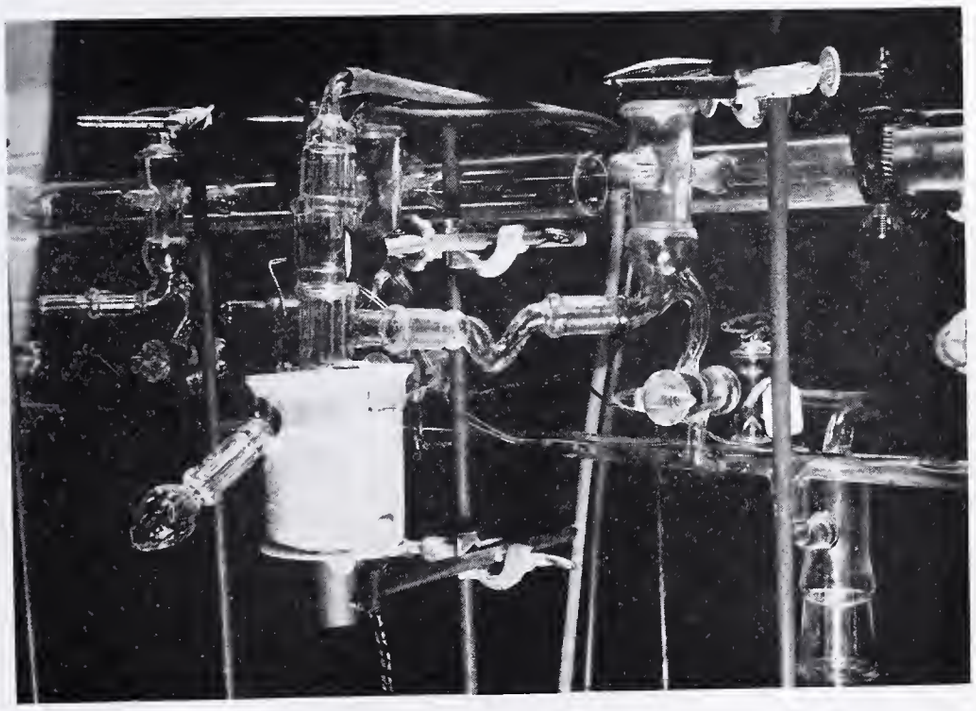

FIGURE 4. STILL AND HEATER ARRANGED FOR OPERATION

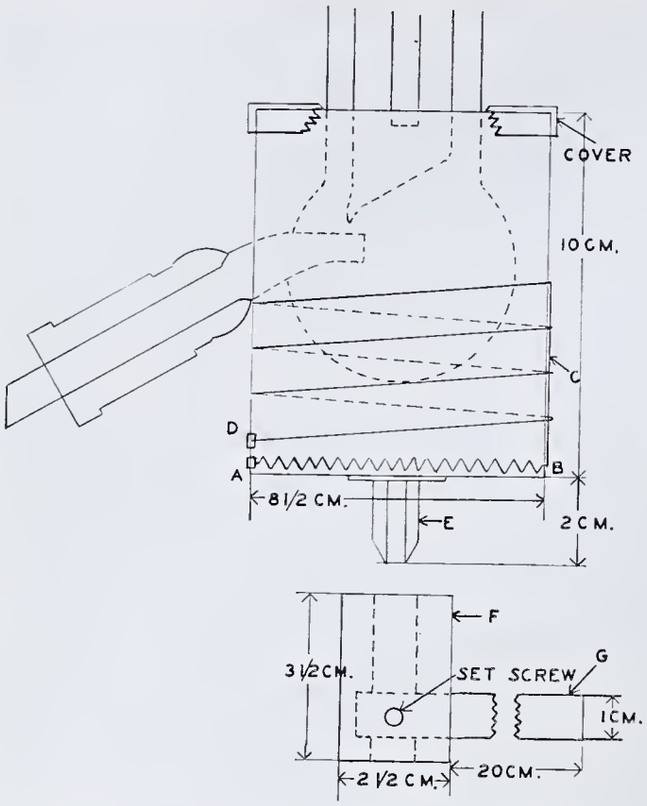

FIGURE 5. DETAILS OF HEATER ASSEMBLY

The cold finger is provided with a drip tip which allows the distillate to drop into an internal receiver. Air, water, or steam may be circulated through the cold finger, depending upon the viscosity and stability of the material being distilled. During the process of degassing, the drip tip is turned away from the receiver, so that the distilland may reflux and in this way ensure complete removal of dissolved or occluded gases, after which the condenser can be rotated so that the drip tip is over the receiver without breaking the vacuum or interrupting the heating. Thus, mild bumping during the degassing process causes no trouble. The receiving flasks are also provided with ₮ joints which allow ready interchangeability if fractionation is desired. A third ₮ joint of suitable size serves to connect the still to the evacuating apparatus. Stills with capacities varying between 1 and 15 ml. have been constructed.

A small modified retort (Figure 8, *A*) has proved adequate for the purification of small amounts of viscous material. This still is used together with a Z-tube (Figure 8, *B*). The Z-tube is placed between the still and the vacuum system and prevents the escape of any of the distillate which normally collects in the small bulb midway between the flask and the joint. It also allows the retort to be adjusted to any desired angle from the horizontal. Removal of the distillate is accomplished by inverting the distillation tube in a small flask filled with a suitable solvent. These are then placed in a desiccator and partially evacuated; when the desiccator is opened the increase in pressure forces the solvent to rise into the bulb containing the distillate. This washing may be repeated as often as necessary. A micro filter bell may be substituted for the vacuum desiccator.

A type of still applicable for sublimation of small quantities of material is shown in Figure 9. A small well at the bottom of the still concentrates the sublimate at the bottom of the cold finger, preventing condensation on the outside walls of the still and on the upper part of the condenser.

Many of the stills and sublimation apparatus described in the literature (*1*, *2*, *4*) may be adapted for use with this apparatus when provided with a ₮ joint of suitable size.

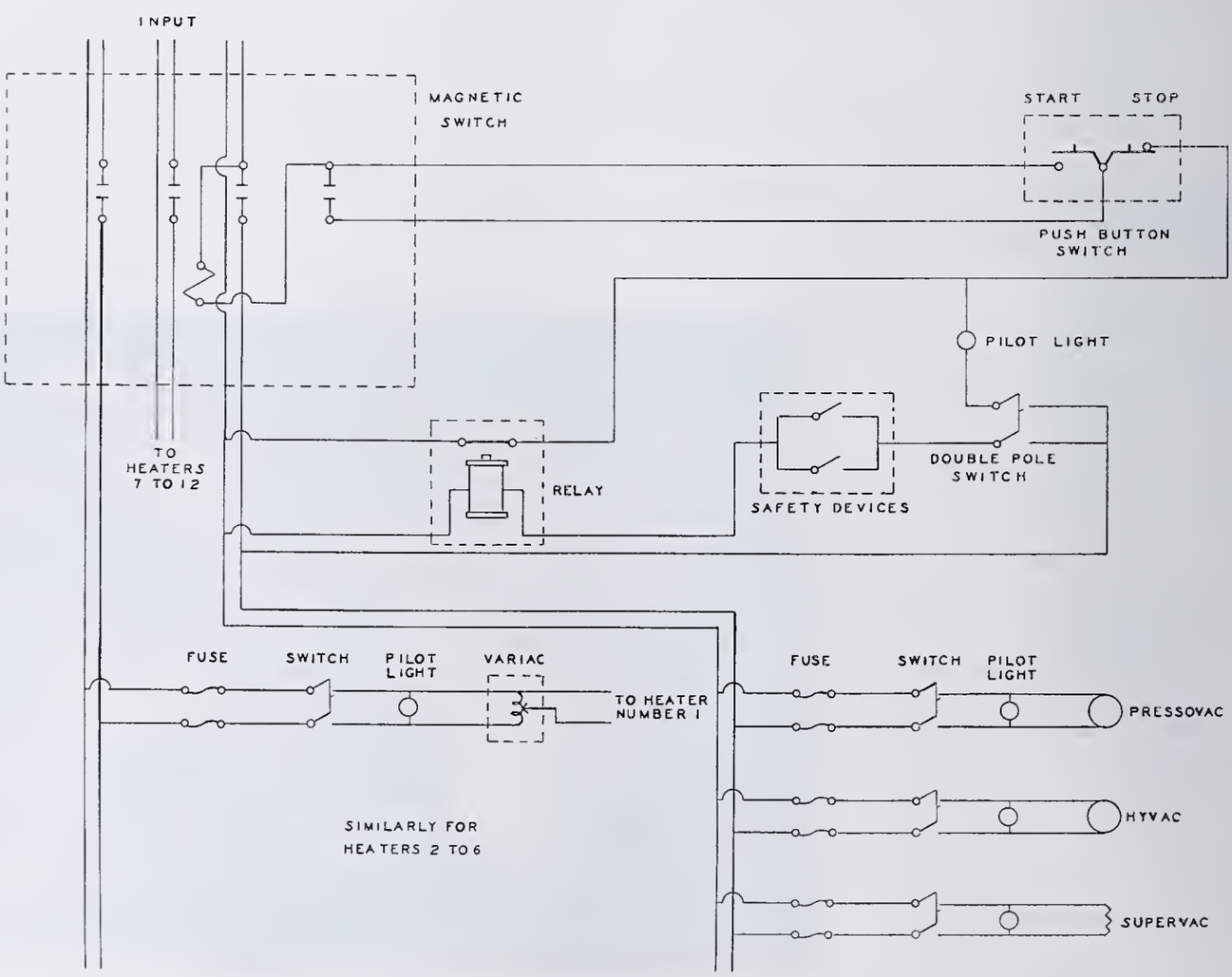

FIGURE 6. WIRING DIAGRAM

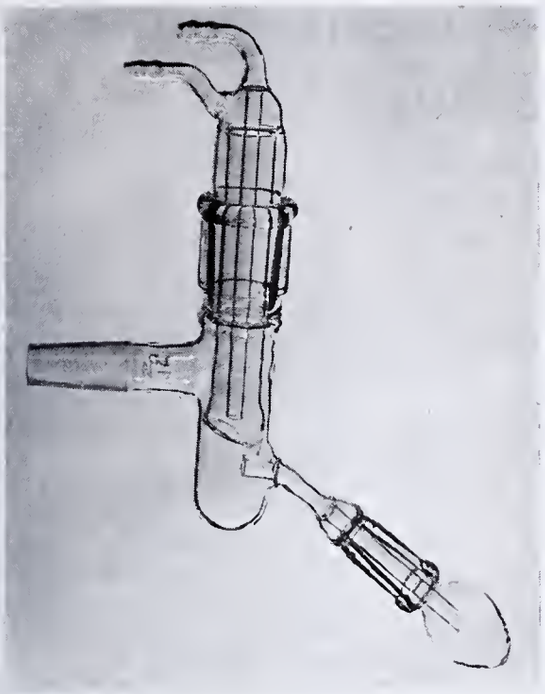

FIGURE 7. IMPROVED TYPE OF MOLECULAR STILL

The ₮ joints on the stills described in this paper have been attached so that the distillates cannot become contaminated with the lubricant on the joint. A heavy vacuum grease with a rubber base has been used on the ground-glass joints which become warm and a lighter grease of the same type has been used on the stopcocks.

FIGURE 8. STILL FOR SMALL AMOUNTS OF VISCOUS MATERIAL (*A*) AND Z-TUBE (*B*)

The apparatus has been in continuous operation for the past year in this laboratory. During this period a number of compounds of high molecular weight, such as cholestenone, dehydroandrosterone, methyl 6-methoxy-*bisnor-i*-cholenate, 3′-alkyl substituted cyclopentenophenanthrenes, and β-keto adipic ester, have been purified by sublimation and distillation. A number of separations such as vitamin K_1 from alfalfa leaf meal oil and testosterone propionate from sesame oil, have been made. The latter was a commercial preparation from which 92 per cent of the desired compound was obtained in a perfectly pure state. During this period it has been found advantageous to clean the traps and regrease the stopcocks approximately every 2 months. The mercury in the condensation pumps becomes only slightly fouled. The lines, however, have remained completely

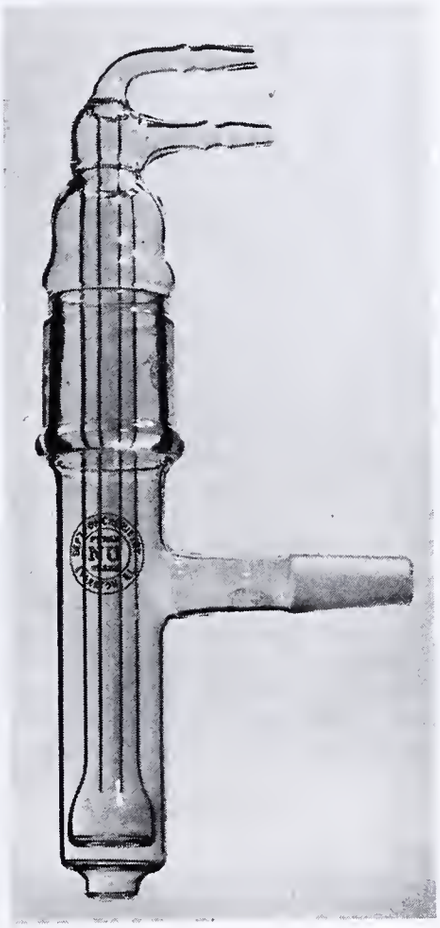

FIGURE 9. SUBLIMATION APPARATUS USEFUL FOR SMALL AMOUNTS

free of contamination. By reducing the vibration of the backing pumps it has been found that the para-rubber connections last almost indefinitely. The frequent and successful use of this apparatus by a number of operators has justified the effort and expense of construction.

Acknowledgment

The authors wish to thank M. N. States of the Central Scientific Company for technical advice and James Morris for the glass work on the apparatus. To one of the referees, K. C. D. Hickman, the authors wish to express their appreciation for suggestions incorporated in the paragraph on the measurement of pressures.

They also wish to thank the Abbott Laboratories, the Glidden Company, the Rockefeller Foundation, and the Upjohn Company for research grants.

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A Viscometric Method for Determining Free Menthol in Peppermint Oil

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SINCE the importation of menthol from the Far East has been cut off by the war, it has become necessary to turn to American peppermint oil as a source. Formerly American oil was used only as a flavoring agent and in medicine, and so was bought on the basis of odor, taste, and appearance. Now, however, if it is to be processed for menthol its purchase price should depend on menthol content and there should be a rapid and reasonably accurate method for menthol assay. Since the United States Pharmacopoeia method (*5*) requires rather more equipment and time than are usually at the disposal of the buyer, a short viscometric method which is applicable to fresh or well-preserved oils has been developed. Dowzard (*3*) suggested viscosity measurements as a means of testing the identity and purity of volatile oils, but did not use it as a means of determining menthol in peppermint oils.

Peppermint oil is composed principally of menthol, menthyl esters, menthone, and hydrocarbons, all of which have approximately the same molecular weight and which, if only molecular weight were considered, might be expected to have about the same effect on the viscosity of the oil. However, since menthol is an alcohol, its hydroxyl group gives it an exalted viscosity, and consequently this property of the oil should depend to a great extent upon the amount of menthol present.

TABLE I. COMPARISON OF VISCOSITIES OF THE PRINCIPAL CONSTITUENTS OF PEPPERMINT OIL

	Seconds
Menthol	1077.2
Menthone	151.8
Menthyl acetate	210.4

Experimental

When menthol, menthone, and menthyl acetate were allowed to drain from an Ostwald pipet at 50° C., the results shown in Table I were obtained. A sample of 3 ml. was used for each determination.

Thus it appeared that the concentration of free menthol might govern the viscosity of peppermint oils, especially since this compound, together with menthone and menthyl esters, constitutes by far the greater part of the whole oil. This was tested with oils whose compositions were adjusted by additions of the pure substances. The results, all at 30° C., are given in Table II.

It will be noted from Table II that oils A, B, and E had approximately the same composition and did not differ markedly in viscosity as evidenced by time of drainage of the pipet. Since the densities of peppermint oils vary but little, viscosities were considered as proportional to times of drainage. The viscosity of oil H was much lower than that of oils A, B, and E, though this oil differed from them substantially only in its free menthol content. Thus the profound effect of a small amount of free menthol on the total viscosity was demonstrated.

Oils B, C, and D illustrate the influence of varying amounts of menthone. Substantial changes in the concentration of this constituent influenced the viscosity only slightly. Since the variation in menthone content of the whole natural oils is well within the range represented in these data, the menthone effect can be neglected.

Oils E, F, and G show the effect of different ester contents. The results indicated that esters have a slightly greater effect on the viscosity than does menthone. However, these oils cover a range of ester concentration much greater than that found in natural oils. Therefore, the slight effect on the viscosity caused by the relatively small variation in ester content in natural oils may be ignored.

In order to calibrate the pipet and establish a basic reference curve for analytical purposes, a sample of peppermint

TABLE II. EFFECT OF COMPOSITION OF OIL ON VISCOSITY

Oil	Composition of Oil: Free menthol	Esters	Menthone	Time of Drainage at 30° C.
	%	%	%	*Sec.*
A	46.55	4.95	32.00	469.2
B	46.30	4.97	32.12	451.6
C	46.70	4.98	24.60	467.6
D	46.35	4.95	45.50	496.4
E	46.06	5.00	30.30	460.2
F	46.15	9.22	30.30	483.4
G	46.00	16.22	30.30	491.0
H	42.29	4.77	32.92	417.2

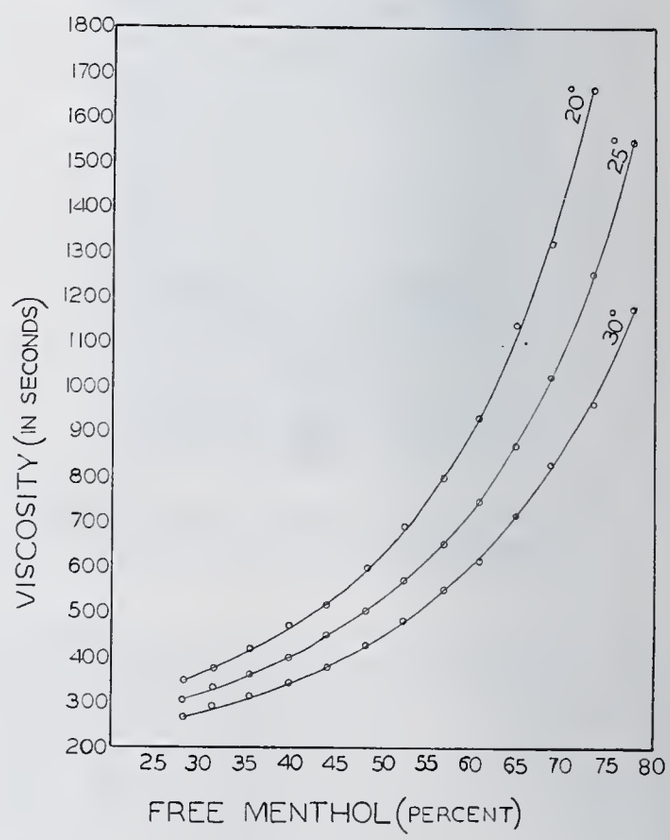

FIGURE 1

Table III. Analysis of Peppermint Oils by Viscometric Method

Oil No.	Viscosity Time at 30° C.	Viscometric Method: Uncorrected, free menthol	Viscometric Method: Turbidity index	Viscometric Method: Corrected, free menthol	Chemical Method, Free Menthol	Difference between Viscometric and Chemical Methods
	Sec.	%		%	%	%
1	561.6	57.0	C	51.7	49.7	+2.0
2	394.4	44.5	A	41.2	41.1	+0.1
3	444.2	49.2	A	45.9	46.2	−0.3
4	464.0	50.8	C	45.5	47.9	−2.4
5	578.4	58.0	A	54.7	55.5	−0.8
6	483.6	52.4	C	47.1	47.0	+0.1
7	583.2	58.3	C	53.0	53.1	−0.1
8	391.0	44.2	A	40.9	40.2	+0.7
9	505.4	53.5	A	50.2	50.3	−0.1
10	564.6	57.2	C	51.9	50.3	+1.6
11	468.0	51.1	A	47.8	47.3	+0.5
12	437.6	48.7	A	45.4	45.6	−0.2
13	471.8	51.5	C	46.2	44.3	+1.9
14	476.6	51.8	A	48.5	46.7	+1.8
15	563.0	57.2	A	53.9	55.0	−1.1
16	631.0	60.5	A	57.2	59.0	−1.8
17	701.0	63.5	A	60.2	59.5	+0.7
18	681.0	62.5	A	59.2	60.8	−1.6
19	385.2	43.7	C	38.4	36.9	+1.5
20	458.4	50.4	A	47.1	46.9	+0.2
21	451.0	49.7	A	46.4	46.1	+0.3
22	532.6	55.1	A	51.8	52.6	−0.8
23	425.8	47.7	A	44.4	44.7	−0.3
24	477.4	51.8	A	48.5	50.8	−2.3
25	426.6	47.6	A	44.3	45.6	−1.3
26	385.6	43.7	A	40.4	41.8	−1.4
27	379.4	43.4	A	40.1	41.2	−1.1
28	434.8	48.4	C	43.1	43.0	+0.1
29	489.6	52.8	A	49.5	51.4	−1.9
30	495.0	53.2	A	49.9	49.5	+0.4
31	566.2	57.3	A	54.0	54.0	0.0

oil was distilled with steam. The first quarter of the distillate, which was low in free menthol (27.9 per cent), was adjusted by adding different amounts of pure 1-menthol to give a series of mixtures covering a wide range in concentration of this constituent. The viscosity of each oil, expressed in seconds, was measured at 20°, 25°, and 30° C. in the same Ostwald pipet used in the above experiments.

When percentage of free menthol was plotted against viscosity in seconds, the regular curves shown in Figure 1 were obtained. However, when natural oils of known free menthol content were allowed to drain from the pipet at one of the temperatures used in the calibration and the time applied to the appropriate curve, it was found that the corresponding free menthol percentage obtained was somewhat higher than the chemical value (Table III). Thus it appeared that natural oils contained a varying amount of some substance other than menthol which affected the viscosity substantially. Determination of resins showed that these substances accounted only in part for the discrepancy.

The interfering substance was correlated with the insolubility of the oil in a mixture of equal parts by volume of methanol and 70 per cent ethanol. The various degrees of turbidity produced when the oil was mixed with four volumes of the solvent mixture are very easily distinguished. They were designated by letters and to each letter was assigned a numerical correction factor as follows:

	Turbidity Designation	Correction Factor
A	Clear to opalescent	3.3
B	Cloudy	4.5
C	Very cloudy to partly insoluble	5.3

The following formula indicates the use of these factors:

Apparent % free menthol from curve − turbidity correction factor = true % free menthol

The menthol contents of a number of natural peppermint oils were determined by both the viscometric and chemical methods (Table III). These oils were from the 1941 crop but were stored in 4-ounce tins sealed at the still. The absence of color in these oils indicated that they were in excellent condition. The samples were measured directly from the tins into the Ostwald pipet without preliminary treatment of any kind.

The results of the analysis of 31 different oils show reasonably good agreement between the chemical and viscometric methods. The greatest difference is 2.4 per cent free menthol in the case of oil 4. However, most of the values are in much closer agreement and the accuracy of the viscometric method is sufficient to give it wide practical application.

Discussion

The viscometric method for the determination of free menthol in peppermint oils was developed primarily for use in the field. The equipment required is simple and inexpensive and can be used under conditions in which the chemical method is impracticable. The use of this method will effect a great saving in time, since a determination of free menthol can be made in less than 20 minutes. The technique is easily acquired and a knowledge of chemistry on the part of the operator is not necessary. The accuracy of the method is sufficient for field purposes and for many applications in the laboratory where extreme accuracy is not essential. A further advantage of the viscometric method is that a free menthol determination can be made on very small samples (2 to 3 ml.) of oil. The sample can be recovered for other determinations.

This method should facilitate work in the breeding of mints for high menthol content, since the breeder will be able to make his own analyses. Since only small samples are required, the use of the viscometric method will materially lessen the number of plants to be grown before selections are made. The rapidity with which results can be obtained by the viscometric method should facilitate the marketing of peppermint oils on the basis of their free menthol content.

The menthol content of the oil furnishes valuable information concerning the proper time to harvest mint for maximum menthol yield (*4*). In the past it has not been possible to analyze sufficient samples so that all growers could have the benefit of this information. The viscometric method, which is both rapid and simple, should be of great help in solving this problem.

The application of the viscometric method is limited to fresh or well-preserved natural oils of normal composition. It is not applicable to oils of high resin content because the resins greatly increase the viscosity of the oil. However, the chemical method also gives unreliable results when preformed resin content is high (*4*) as well as when heating during saponification causes resinification to take place (*1*, *2*). The viscometric method can be used only to determine free menthol. In order to obtain total menthol, it is necessary to use the chemical method for the determination of the ester value, but, in many cases, a determination of free menthol gives all the information desired.

Acknowledgment

The authors are indebted to N. K. Ellis, of the Horticulture Department, Purdue University Agricultural Experiment Station, for the peppermint oil samples used in this work. It is largely due to his stimulus that this work was undertaken.

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Thixotropic Behavior of Oils

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Various types of oils in the viscosity range of 1 to 800 poises were measured on a rotational viscometer capable of imparting shearing stresses over a wide range. From these oils, flow curves were obtained extending from low to high rates of shear. All these oils showed a definite thixotropic behavior and exhibited all the characteristics of thixotropic plastics above a certain rate of shear designated as "the limiting rate of shear". Below this critical point the oils behaved like true Newtonian liquids, showing no signs of thixotropic structure. The limiting rates of shear were found to be related to the measured true Newtonian viscosities of the oils. The product of limiting rate of shear and viscosity was a constant for all the oils tested.

OILS have been generally assumed to be true Newtonian liquids, even to the extent that industrial laboratories are satisfied to determine the viscosity of an oil by a one-point measurement. It has been suggested by some workers in the field that certain oils exhibit a pseudoplastic characteristic.

Extensive studies in this laboratory have shown that the many different oils investigated are not pseudoplastics but are true Newtonian liquids for a limited range of rate of shear and behave, beyond the limited range, at least for the heavier oils, like thixotropic plastics.

In the literature (*2*, *9*) thixotropy is defined as an isothermal gel-sol-gel transformation. Thixotropic behavior does not require a complete transformation; it is considered a sufficient condition for the presence of thixotropy that a material changes its plastic viscosity from a higher to a lower value as a result of mechanical agitation and regains its original high viscosity upon rest. However, the thixotropic characteristic of a material is also a function of time, which means that the viscosity of the thixotropic material depends not only on previous mechanical agitation but also on the time period during which the material has been subjected to such mechanical agitation.

Thixotropy is found in paints (*8*, *10*, *11*), where it often proves to be useful, in bentonite suspensions (*7*), in gelatin sols (*8*), in iron oxide sols (*13*), and in printing inks (*3*). Little or no reference has been made to the thixotropy of oils. This thixotropic characteristic of oils probably has not been recognized because most instruments used for viscosity measurements have not permitted the application of sufficiently high rates of shear, or only one-point viscosity determinations have usually been performed, and these are insufficient to show thixotropy. The rotational viscometer developed in this laboratory, described by Green (*3*), was well suited for studying the thixotropic behavior of oils.

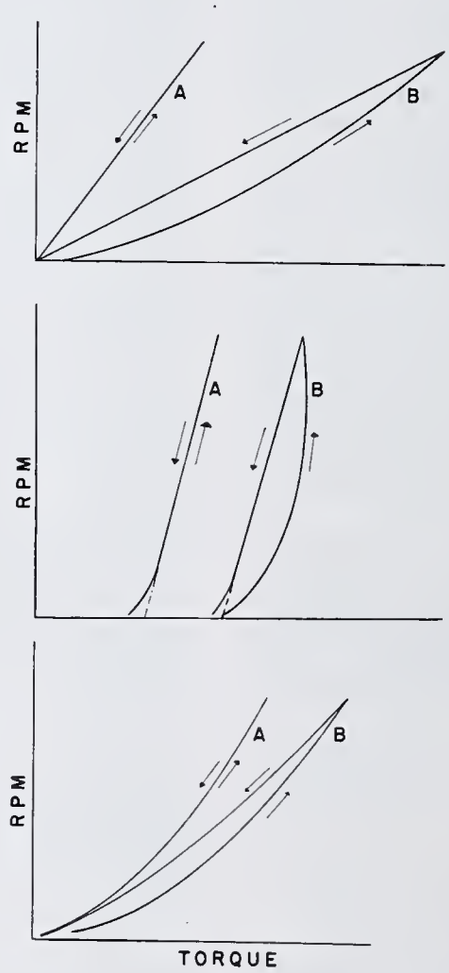

FIGURE 1. FLOW CURVES

Upper. *A.* Nonthixotropic true Newtonian liquid
B. Thixotropic true Newtonian
Center. *A.* Nonthixotropic plastic
B. Thixotropic plastic
Lower. *A.* Nonthixotropic pseudoplastic
B. Thixotropic pseudoplastic

Instrument

The viscometer has played an important part in obtaining the results reported below. It is built on the rotational principle, where the cup is rotated at various speeds and the bob is stationary, being suspended from a helical spring. The torsional modulus of this spring is calibrated by weights. Various springs are used to cover a wider range of viscosity measurements. The dimensions of the cup and the bob have been chosen to minimize the effect of plug flow and turbulence. A lid on the cup prevents the oils from climbing up the shaft of the bob and from being thrown out of the cup at higher rates of shear. No "channeling" could be evidenced, provided the bob was perfectly in center with the cup. The end effect introduced by the bottom of the bob is about 2 per cent of the whole shearing effect and therefore can be disregarded in calculating viscosities and yield values. A constant temperature bath keeps the temperature of the material to be investigated within ±0.2° C.

Flow Curves

The viscosities and the yield values of the different materials are obtained from flow curves, found by plotting the number of revolutions per minute as a function of the resulting torque. In the process of getting such flow curves, the speed of the cup is changed and the corresponding deflection of the bob, a measure of the torque, is marked down. It is understood that the amount of deflection depends upon the angular velocity of the cup. Since the angular velocities or

the revolutions per minute are proportional to the rate of shear or to the velocity gradient, in the flow curve diagram, the revolutions per minute of the ordinate may be replaced by a rate of shear ordinate. The relationship between the revolutions per minute (r. p. m.) and velocity gradient or rate of shear (dv/dr) in sec.$^{-1}$ for a rotational instrument is

$$1 \text{ r. p. m.} = 60\, r^2 hS dv/dr \tag{1}$$

where r is any radius between the cup and the bob, h is the immersed height of the bob, and S is an instrumental constant equal to

$$(1/R_c^2 - 1/R_b^2)/4\pi h$$

where R_c is the radius of the cup and R_b is the radius of the bob.

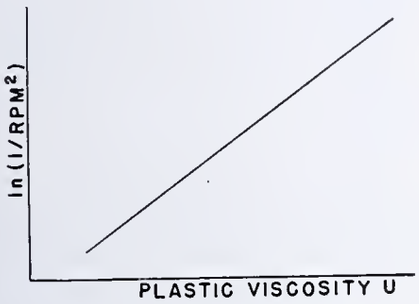

FIGURE 2. SCHEMATIC CURVE SHOWING CHANGE IN PLASTIC VISCOSITY WITH TOP R. P. M. FOR THIXOTROPIC PLASTIC

The mean rate of shear can be calculated for a given r. p. m. by substituting a mean radius in Equation 1. Then the equation for the mean rate of shear expressed in reciprocal seconds is

$$dv/dr = 4 \text{ r. p. m.}/60\, hS\, (R_c + R_b)^2 \tag{2}$$

Though the measurements were made with cups and bobs of various sizes, the reported revolutions per minute were recalculated for a cup of 1.5-cm. radius and a bob of 1.3-cm. radius and 5.1-cm. immersed height. Then, the relationship between the revolutions per minute and the mean velocity gradient or the mean rate of shear (dv/dr) in sec.$^{-1}$ is

$$1 \text{ r. p. m.} = 1.36\, (dv/dr) \tag{3}$$

The torque, also called the shearing stress, may be expressed in dynes-centimeter, a value which can be obtained by multiplying the deflection by the torsional constant of the helical spring.

Flow curves are obtained by increasing the rates of shear to any desired maximum value and then decreasing them until the starting value is reached. Following this procedure hysteresis loops (*3, 6, 8, 10*) are obtained for thixotropic materials, while in nonthixotropic materials the up- and downcurves coincide. In Figure 1 six typical flow curves are shown. *A* (upper) is representative of a nonthixotropic true Newtonian liquid; the up- and downcurves coincide and form a straight line passing through the point of origin. *B* (upper) is representative of a thixotropic liquid; its upcurve has a continuous curvature, while its downcurve is again a straight line passing through the point of origin. *A* and *B* (center) are comparable to *A* and *B* (upper), but are obtained from true plastic materials and are representative of such substances. The downcurve of a true plastic material has a large linear portion, but at lower rates of shear shows a development of some curvature, explained (*1, 4, 12*) as caused by plug flow. Experimental data have been given by Green (*3*). However, the curvature in most cases where pigment suspensions and oils were investigated shows a greater extension than would be expected from plug flow. This extended curvature at lower rates of shear is believed to be caused mostly by thixotropy. The plastic viscosities and the yield values relating to the straight portion of the downcurve may be obtained from Reiner's equation as follows:

$$U = \frac{T - T_2}{\omega} S \tag{4}$$

$$f = T_2 C \tag{5}$$

where U is the plastic viscosity in poises and T is the torsion in dynes-centimeter. T_2 is the torsion corresponding to the intercept which is obtained by extending the straight portion of the flow curve to the torsion axis, ω is the angular velocity, f is the yield value in dynes per square centimeter, and C is an instrumental constant equal to $S/\ln(R_c/R_b)$.

A (Figure 1, center) represents a flow curve obtained from a nonthixotropic true plastic, and it is characterized by the fact that the up- and downcurves coincide. The criterion of any plastic is the presence of an intercept of the downcurve with the torsion axis, indicating the existence of yield value. *B* (center) is a flow curve obtained from a thixotropic true plastic, where the upcurve has a curvature throughout all rates of shear and does not coincide with the downcurve.

Finally (Figure 1, lower) two flow curves are shown, obtained from pseudoplastic materials. *A* represents a nonthixotropic and *B* a thixotropic material. Such pseudoplastic flow curves are characterized by the fact that even their downcurves have a curvature throughout all speeds.

Thixotropy of Oils

Any thixotropic plastic will show a viscosity which depends upon the highest rate of shear to which the material has been subjected before starting on the downcurve. These downcurves are always found to be straight lines, thus indicating a stable condition. This stable condition has been termed thixotropic level by Green (*3*) and can be identified by its top rate of shear or top r. p. m.

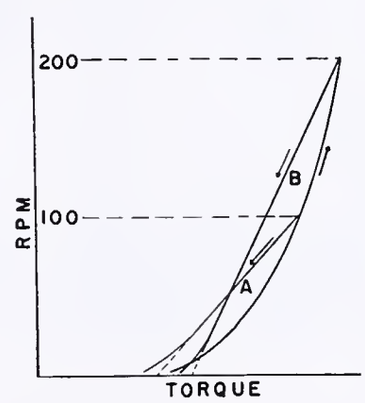

FIGURE 3. FLOW CURVES
Hysteresis loops obtained for thixotropic plastic measured to top r. p. m. of (*A*) 100, (*B*) 200

The correlation found between the plastic viscosity and the respective top rate of shear or top r. p. m. is shown in Figure 2, drawn schematically according to an equation given in another paper (*5*):

$$U = \ln (K/\text{r. p. m.}^2)/m \tag{6}$$

where $2/m$, also designated as M, is the coefficient of thixotropic breakdown and is defined as the loss in shearing force

Table I. Limiting Rates of Shear for Oils of Varying Viscosity (Figure 4)

(Temperature constant, 30° C.)

Oils	Viscosity (True Newtonian)	Limiting Revolutions per Minute (*RPM*)	Limiting Rates of Shear	1/Limiting Rates of Shear	Viscosity × Limiting Rates of Shear
	Poises		*Sec.*$^{-1}$	*Sec.*	*Dynes/sq. cm.*
Mineral oil[a]	780	12	9.0	0.113	6900
Isobutylene oil	770	12	9.0	0.113	6800
Linseed oil (low acid)	380	25	18.4	0.054	7000
Linseed oil	250	38	28.0	0.036	7000
Isobutylene oil	180	52	38.3	0.026	6800
Linseed oil	125	75	55.5	0.018	6800
Linseed oil	115	81	59.8	0.017	6800
Esso lubricant 3000	103	95	70.0	0.014	7200
Mineral oil and gum varnish	72	135	100	0.010	7100
Linseed oil	48	205	151	0.007	7200
Mineral oil	31	310	228	0.004	7100
Mineral oil	30	315	231	0.004	6900
Isobutylene oil	23	405	298	0.003	6800
Mineral oil	21	455	335	0.003	7000
Mineral oil[a]	20	490	360	0.003	7200
Mineral oil	19	500	370	0.003	7000
Linseed oil	16	580	430	0.002	6800
Linseed oil[b]	10	725	532	...	..
Castor oil[b]	4	1450	1070	...	..
Mineral oil[b]	3	1450	1070	...	..
Isobutylene oil[b]	1	1450	1070	...	..
Mineral oil (medical)[b]	1	1450	1070	...	..

[a] Standard viscosity oils supplied by National Bureau of Standards as refined mineral oil.
[b] Since, owing to instrument limitations, limiting rates of shear could not be reached, maximum *RPM* and rates of shear to which oils could be measured are tabulated. At these rates of shear, below limiting rates of shear, oils still behave like true Newtonian liquids.

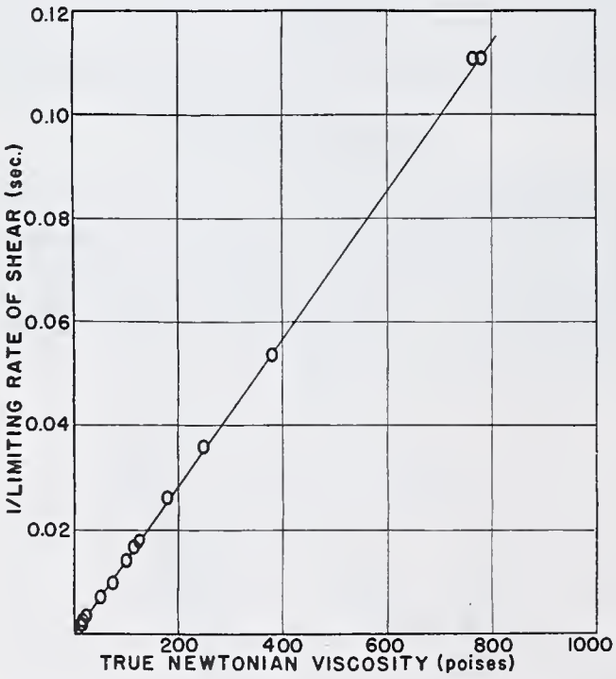

Figure 4. Change of Reciprocals of Limiting Rate of Shear with True Newtonian Viscosities of Oils

per unit area per unit increase in velocity gradient, and K is an integration constant and is constant for each material.

When flow properties of thixotropic materials are investigated, a relationship is found between the intercept on the torque axis and the respective top rate of shear. In most cases the intercept increases with an increase in top rate of shear or top r. p. m., which is shown in Figure 3 schematically. This subject will be treated in more detail by H. Green and the author in a subsequent paper.

The flow curves obtained from the oils seem to be a combination between A (Figure 1, upper) and B (Figure 1, center). The outstanding fact concerning the oils which have been investigated in this laboratory is their apparent normal behavior (true Newtonian) up to a certain rate of shear. Only above this rate of shear, which will be called the "limiting rate of shear", does the thixotropic behavior of oils become apparent. This limiting rate of shear is not a fixed value but depends essentially upon the viscosity of the oil measured in its true Newtonian region. These limiting rates of shear, even for highly viscous oils, are so high that most standard methods of measuring viscosities of oils did not permit using sufficiently high rates of shear to detect thixotropy. These limiting rates of shear are not very sharply defined, but approximate values are given in Table I for various oils. Figure 4 shows the reciprocal of the limiting rates of shear plotted as a function of their true Newtonian viscosities. This relationship is linear, indicated by the straight line.

This indicates that only at extremely large finite values of the viscosity will the limiting rate of shear approach zero. On the other hand, oils of extremely low viscosity, below 1 poise, may have a very high value of limiting rate of shear—indeed, a value so high that it may approach infinity. Though it has not been possible to determine the limiting rate of shear value for oils of low Newtonian viscosities because of limitations imposed by the viscometer, it may very well be that all oils are thixotropic.

The force (torque) acting between two adjacent layers of the oil is equal to the product of viscosity and rate of shear. This product for the limiting rate of shear was found to be approximately constant for all oils (Table I) if the measurements were performed by increasing the rates of shear (or r. p. m.) very fast and by immediately decreasing them without waiting at the top r. p. m. The shearing force acting between

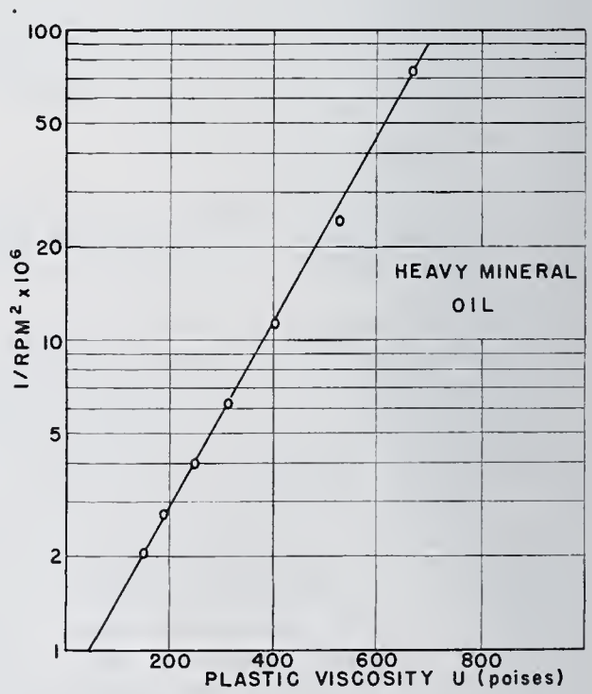

Figure 5. Experimental Curve Showing Change of Plastic Viscosity with Change in Top R. P. M.

Table II. Viscosities of Heavy Mineral Oil

Top Rates of Shear *Sec.*$^{-1}$	Top R. P. M.	Plastic Viscosity *Poises*	Torque Intercept *Dynes cm.*
A. Plastic viscosities and intercepts at various thixotropic levels. Temperature constant, 30° C.			
83	112	665	1.0×10^5
150	205	538	6.7
222	302	402	14.5
290	393	316	20.5
365	495	250	24.0
440	595	197	28.0
515	700	150	31.0

B. True Newtonian viscosities at various temperatures. Top r. p. m. below "limiting *RPM*"

Temperature ° *C.*	True Newtonian Viscosity *Poises*
25	1130
30	780
40	290
50	130
60	60
80	15
90	10

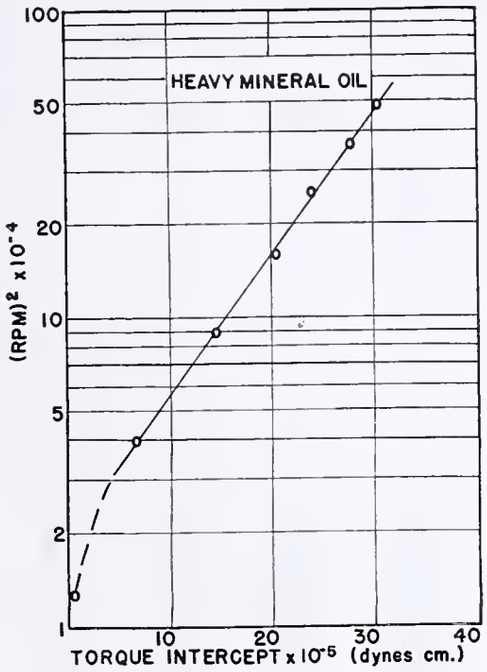

Figure 6. Experimental Curve Showing Change of Intercept with Change in Top R. P. M.

two adjacent layers of the oil calculated from the average value of the product of viscosity and limiting rate of shear was found to be about 7000 dynes per square centimeter. This minimum shearing force of 7000 dynes per square centimeter may be required to overcome an energy barrier before the alignment of the micelles in the direction of rate of shear can start.

Little has yet been said about the flow curves of oils above the limiting rates of shear. In this region these flow curves are very much like flow curves obtained from true plastic materials; in fact, an intercept can be found for the downcurves of the hysteresis loops of oils. These downcurves contain fairly large straight-line portions very similar to the straight-line portions of downcurves of true plastics.

Measurements furthermore indicate that thixotropic levels exist which control the plastic viscosity of the oil as a function of the top rate of shear or top r. p. m. Figure 5 and Table II, A, show the relationship of the plastic viscosity of a typical oil to the respective top r. p. m. Any doubt regarding the plasticity of oils above the limiting rates of shear is removed by the similarity of Figures 2 and 5.

But there is further indication that oils can be identified as true plastics above the limiting rates of shear, since in accordance with expectation the intercept increases with an increase in top rate of shear, or top r. p. m., as shown in Figure 6 and Table II, A. Measurements also show that a complete recovery of structure takes place if the oils are left at rest for a period of time following shear agitation. Since this is one of the most important characteristics of thixotropy, it bears out the statement that oils show thixotropic behavior above their limiting rates of shear.

Table III. Heavy Mineral Oil Flow Curves Measured to Various Top R. P. M. (Figure 7)

(Temperature constant, 30° C.)

R. p. m.	Upcurve Torque $\times 10^{-5}$ *Dynes cm.*	Downcurve Torque $\times 10^{-5}$ for Various Thixotropic Levels: 112 r. p. m.	205 r. p. m.	302 r. p. m.	393 r. p. m. *Dynes cm.*	495 r. p. m.	595 r. p. m.	700 r. p. m.
14.5	..	4.6	4.25	3.55	3.36	3.0	2.48	2.3
22	..	6.5	6.0	5.7	4.95	4.6	3.9	3.5
36	10.6	11	10.6	9.7	9.0	8.1	7.2	6.5
63.5	18.4	18.8	18.0	16.6	...	..	...	..
76	..	..	..	...	18.6	16.6	...	13.4
85	25.8	25.8	24.8	23.3	...	..	17.4	..
112	34	..	31.9	29.9	27.6	24.9	...	..
130	39.9	..	36.6	36.4	...	..	27.2	24.2
145	..	..	...	...	36.1	32.9	...	..
158	46.0	..	44.2	41.5	...	..	...	..
180	52.3	..	50	47	43.9	39.7	35.9	..
192	..	..	...	...	...	..	...	33.9
205	56.0	..	...	51.9	...	..	...	..
218	..	..	...	...	52.0	46.0	...	..
234	60.9	..	...	56.7	...	..	43.5	..
252	64	..	...	61	57	53.0	...	42.4
278	67.5	..	...	64.9	...	..	52	..
289	..	..	...	...	62.5	57.0	...	..
302	70	..	...	...	...	..	...	..
312	..	..	...	...	66	..	...	51.5
325	73	..	...	...	...	61	57	..
349	74.8	..	...	...	...	..	...	..
360	..	..	...	...	...	65	...	..
370	76	..	...	...	74.0	..	61	56.5
393	77.8	..	...	...	...	69.1	...	..
414	..	..	...	...	...	..	65.2	..
420	80.2	..	...	...	...	..	...	..
423	..	..	...	...	...	72.8	...	59.8
445	80.2	..	...	...	...	..	...	..
454	..	..	...	...	...	75.5	69	..
467	80.7	..	...	...	...	..	...	..
474	..	..	...	...	...	..	...	64.3
495	81	..	...	...	...	..	73	..
520	82.3	..	...	...	...	..	...	67.5
523	..	..	...	...	...	..	75.9	..
544	81.9	..	...	...	...	..	...	..
568	81.7	..	...	...	...	..	...	..
569	..	..	...	...	...	..	79	71
595	81.5	..	...	...	...	..	...	..
621	81.5	..	...	...	...	..	...	73.8
648	81	..	...	...	...	..	...	..
668	..	..	...	...	...	..	...	76.3
676	80.5	..	...	...	...	..	...	..
700	79.3	..	...	...	...	..	...	..

TABLE IV. HEAVY MINERAL OIL TORQUE-TIME CURVES AT VARIOUS CONSTANT R. P. M. (FIGURE 8)

(Temperature constant, 30° C.)

Time	Torque × 10^{-6} for Various Thixotropic Levels					Time	Torque × 10^{-6} for Various Thixotropic Levels				
	73 r. p. m.	140 r. p. m.	200 r. p. m.	300 r. p. m.	400 r. p. m.		73 r. p. m.	140 r. p. m.	200 r. p. m.	300 r. p. m.	400 r. p. m.
Sec.						*Sec.*					
0	2.67	4.55	6.09	8.23	10.6	130	2.43	3.37	3.84	5.78	6.43
3	..	4.54	5.12	7.09	...	140	2.41	3.35	3.8	5.72	6.33
4	2.61	..	..	..	8.5	150	2.4	3.34	3.77	5.7	6.28
7	2.6	4.35	4.81	..	..	160	2.39	3.3	3.75	5.61	6.19
8	..	..	..	6.9	8.14	170	2.38	..	3.71	5.6	6.12
10	..	..	4.67	..	..	180	2.37	3.26	3.67	5.56	6.08
11	2.59	3.92	..	..	..	190	2.36	..	3.65	5.5	6.01
13	..	..	..	6.83	7.9	200	2.35	..	3.63	5.48	5.97
14	..	..	4.56	..	..	210	2.35	3.18	3.61	5.41	5.91
15	2.58	3.83	..	..	..	220	2.34	..	3.57	5.4	5.88
18	..	..	..	6.77	7.8	230	2.33	..	3.54	5.38	5.83
19	..	3.8	4.49	..	..	240	2.32	3.13	3.52	5.33	5.8
20	2.58	..	..	..	..	270	2.3	3.08	3.45	5.27	5.7
23	..	3.77	..	6.72	..	300	2.28	3.05	3.4	5.2	5.61
24	..	..	4.39	..	..	330	2.26	3.01	3.35	5.13	5.54
25	2.57	..	..	..	7.68	360	2.28	2.98	3.34	5.11	5.49
28	..	3.75	..	..	..	390	2.24	2.94	3.3	5.08	5.46
29	..	..	4.39	..	..	420	2.21	2.91	3.28	5.05	5.4
30	2.56	..	..	6.67	7.63	450	2.2	2.9	3.26	5.02	5.38
34	..	..	4.35	..	..	480	2.2	2.87	3.23	5.0	5.33
35	2.56	3.71	..	6.58	7.58	510	2.2	2.85	3.2	4.98	5.32
40	2.55	3.68	4.32	6.53	7.51	540	..	2.84	3.19	4.98	5.3
50	2.51	3.65	4.22	6.44	7.38	570	..	2.84	3.15	4.95	5.3
60	2.51	3.61	4.16	6.33	7.21	600	..	2.8	3.14	4.95	5.28
70	2.5	3.57	4.1	6.23	7.1	630	..	2.8	3.11	4.94	5.28
80	2.49	3.54	4.04	6.12	6.95	660	..	2.78	3.1	4.92	5.28
90	2.48	3.5	4	6.04	6.81	690	..	2.76	3.08	4.92	5.27
100	2.47	3.47	3.95	5.98	6.71	720	..	2.73	3.06	4.92	5.27
110	2.45	3.43	3.91	5.91	6.61	750	..	2.73	3.05	4.92	5.27
120	2.44	3.4	3.89	5.82	6.51	780	..	2.71	3.05	4.92	5.27

Experimental Curves

A large number of oils have been measured at various top r. p. m. Since all are of a similar nature, only one representative flow curve is shown in Figure 7 and Table III.

Figure 8 and Table IV show the decrease in torque as a function of time for constant rates of shear or r. p. m. This decrease is typical for thixotropic plastics.

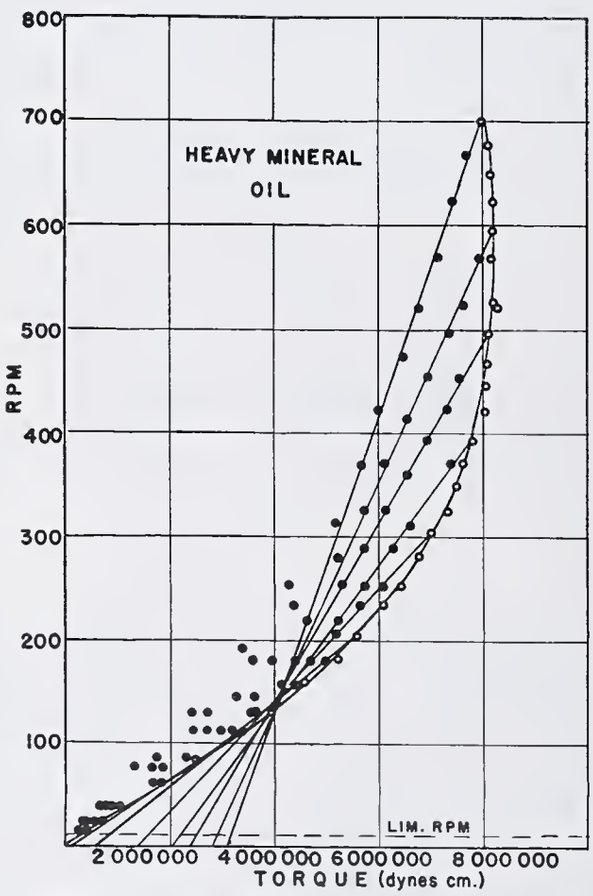

FIGURE 7. EXPERIMENTAL FLOW CURVES OBTAINED FROM VARIOUS TOP R. P. M.

Temperature, Turbulence, and Slippage

It is evident that the type of flow curves obtained from oils is not caused by factors like change of temperature, turbulence, or slippage.

Slippage can be immediately discarded as a result of experiments made with a grooved bob and cup (*3*).

Turbulence can be eliminated as cause for the particular structure of the oil flow curves, since turbulence would have tended to increase rather than decrease the forces (torques) at higher rates of shear.

Finally there remains the question of temperature. Increase of temperature undoubtedly decreases viscosity. The point then is, how much rise in temperature is required to decrease the oil viscosity to the same plastic viscosity obtained by increasing the rate of shear. To determine this, a heavy mineral oil was chosen and its viscosity below the limiting rate of shear was measured at various temperatures. Though the decrease in viscosity is substantial, it is not large enough to account for the rapid decrease in viscosity with an increase in top rate of shear. In Table II the true Newtonian viscosities are given for various temperatures and the plastic viscosities are given for various thixotropic levels and are designated by their respective top rates of shear and top r. p. m.

Table II makes it obvious that the decrease in plastic viscosity resulting from an increase in top rate of shear is too great to be entirely caused by an increase in temperature.

This point is more clearly shown by plotting the plastic viscosities at various top r. p. m. against the true Newtonian viscosities obtained at various temperatures (Figure 9 and Table V). For example, an increase from 100 to 700 top r. p. m. decreases the plastic viscosity from 710 to 150 poises, which in turn requires a temperature increase from 31° to 48° C., if the temperature is responsible for the entire increase in viscosity. However, no appreciable temperature increase could be observed if the temperature was taken before and immediately after the measurement. Therefore temperature can also be ruled out as a determining factor for the particular shape of the oil flow curves.

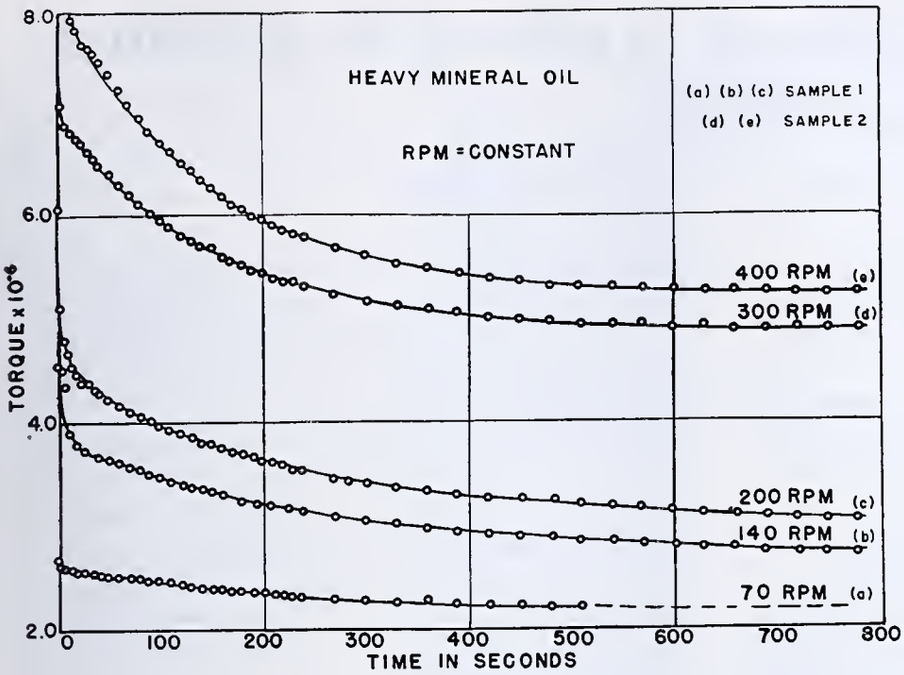

Figure 8. Torque-Time Curves at Various Constant R. P. M.

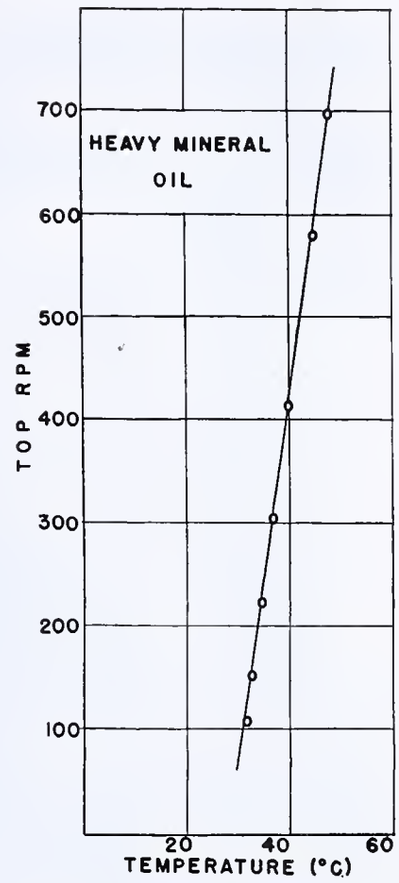

Figure 9. Curve Constructed to Show Increase in Temperature Required to Obtain Same Viscosity as Obtained by Increase in Top R. P. M.

Table V. Interpolated Data from Figure 9

Viscosity *Poises*	Top R. P. M.	Temperature ° *C.*
660	110	31
600	155	32
500	225	34
400	305	36.5
300	415	39.5
200	580	44.5
150	700	48

Conclusions

A full theoretical treatment of the phenomenon of the thixotropic behavior of oils has not yet been developed, but a few suggestions may elucidate the results so far presented.

At rest all molecules are distributed at random, taking on a statistic average position. Applying a rate of shear beyond the limiting rate of shear, which is equivalent to developing a minimum directional force, the molecules may start an alignment in the direction of shear; hence the original random structure of the oil breaks down, as indicated by a decrease in plastic viscosity. Upon rest the molecules will slowly return to their original random position.

Discussion

According to the literature, oils are often recommended for use in calibrating viscometers, but this may lead to a serious error (*3*). The present paper shows that unreliable results may be obtained if oils are used above their limiting rates of shear, since most oils behave like true Newtonians only below this critical point. The danger of an unreliable calibration with oils is particularly great if highly viscous oils are used, since their limiting rates of shear are very low.

However, any such oil can be used for calibration if the applied rates of shear are kept below the limiting rate of shear of the oil, in the range where it behaves like a true Newtonian liquid.

Although the oils tested showed no evidence of impurities, some oils contain waxes and other contaminants; therefore, the question of a possible separation at high rates of shear remains to be discussed. Separation would probably have a decreasing effect on the torque and therefore would show phenomena like those due to a thixotropic breakdown of the oil structure. However, the effects described above are not due to any detectable separation, since repeated measurements after short periods of time yield identical results, and the elapsed time intervals, of a few minutes, are long enough to allow a thixotropic recovery, but not a redistribution of any separated materials. It is highly improbable that a redistribution or redispersion could take place while the material is at complete rest, even if a longer time for recovery were allowed.

Acknowledgment

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Rapid Determination of Water in By-Product Sulfur

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By-product sulfur, sometimes known as sulfur paste, recovered during the purification of manufactured, natural, or refinery gases using liquid purification and sulfur recovery processes, is being used extensively as a fungicide. The texture and consistency of the specially milled and creamed sulfur are controlled by its water content during its production. A rapid density method for the determination of water in the by-product sulfur requires no weighing, but merely comprises liquefying the sulfur paste, deaerating the sample by suction with constant stirring in a special metal container, counterbalancing a given weight, and reading the moisture content directly from a calibrated flask.

Its accuracy has been established by comparison with a distillation procedure previously developed. The rapid density method will give results within 0.5 per cent of the true water content of by-product sulfur. The time required for a determination is 10 minutes.

BY-PRODUCT sulfur, sometimes known as sulfur paste, is recovered during the purification of manufactured, natural, or refinery gases which utilize liquid purification and sulfur recovery processes (*5, 6, 9, 10*). At Rochester, N. Y., an "ammonia-Thylox" liquid purification system is in operation. The details of the process have been described by Bowman (*1*). The chemistry of the process as studied by Gollmar (*5*) and in the author's laboratory can be briefly summarized as follows:

Certain thioarsenates are capable of absorbing oxygen from the air, replacing part of the sulfur in the thioarsenate molecule, and precipitating it as elemental sulfur. This lower sulfur thioarsenate molecule can absorb hydrogen sulfide, forming the original thioarsenate, and the cycle can be repeated continuously. In practice theoretical quantities of arsenic trioxide and soda ash are dissolved in boiling water and added to the system as required to maintain an arsenic trioxide concentration of 6.0 grams per liter. The gas containing some 25.9 grams (400 grains) of hydrogen sulfide per 2.83 cubic meters (100 cubic feet) is scrubbed by the Thylox (arsenic) liquor containing the thioarsenates as indicated in the reactions that follow:

A. Preliminary reactions:

$$As_2O_3 + 2Na_2CO_3 + H_2O \longrightarrow 2Na_2HAsO_3 + 2CO_2 \quad (1)$$

$$2Na_2HAsO_3 + 5H_2S \longrightarrow Na_4As_2S_5 + 6H_2O \quad (2)$$

$$Na_4As_2S_5 + O_2 \longrightarrow Na_4As_2S_5O_2 \quad (3)$$

B. In the hydrogen sulfide absorber the following reactions take place in the ammonia-Thylox process:

$$(NH_4)_4As_2S_5O_2 + H_2S \longrightarrow (NH_4)_4As_2S_6O + H_2O \quad (4)$$

$$(NH_4)_4As_2S_6O + H_2S \longrightarrow (NH_4)_4As_2S_7 + H_2O \quad (5)$$

Reaction 4 is faster than 5 and represents the main reaction in the absorber.

C. In the thionizer the Thylox liquor is treated with air under pressure, where oxygen is absorbed and sulfur liberated as follows:

$$(NH_4)_4As_2S_7 + {}^1/_2O_2 \longrightarrow (NH_4)_4As_2S_6O + S \quad (6)$$

$$(NH_4)_4As_2S_6O + {}^1/_2O_2 \longrightarrow (NH_4)_4As_2S_5O_2 + S \quad (7)$$

Reaction 7 is faster than 6 and is believed to be the main reaction taking place in the pressure thionizer.

When the pH of a solution of $(NH_4)_4As_2S_6O$ drops below 7.3, arsenic trisulfide and free sulfur precipitate out, indicating some reduction of pentavalent As^{+++++} to trivalent As^{+++}.

Figure 1 presents a view of the hydrogen sulfide removal system with the two absorbers at the left, the pressure thionizer on the right, and the operating building in the center. The plant is capable of handling some 566,300 cubic meters (20,000,000 cubic feet) of gas per day. The sulfur slurry released at the top of the thionizer flows to a supply tank, thence to a continuous vacuum filter where the by-product sulfur is separated and washed.

The nature and general properties of by-product sulfur have been described previously (*2, 3, 4, 7, 8*). By-product sulfur consists essentially of water, free sulfur, less than 1 per cent of iron oxide, and less than 1 per cent of water-soluble salts. The by-product sulfur as it comes from the filters consists of a yellow-gray sticky paste containing about 45 per cent of water. To prepare this material for market, the sulfur is milled and creamed in special equipment and the moisture content is raised to 52 to 60 per cent, depending upon the type of product desired. In this condition, it is being used extensively today as a fungicide not only for apple orchards but for other fruits as well.

In preparing the final milled sulfur at the plant, it is necessary to keep the water content within definite limits. This requires a rapid and reliable method for determination of the water in the sulfur paste. The author (*8*) previously described a distillation method for determination of water in by-product sulfur, which was based upon the separation of water from the sulfur by the use of a mixture of light oil and gas oil which aided in distilling over of the water into a calibrated trap using a reflux condenser. This method proved to be accurate and convenient. However, the 2 to 3 hours required for the determination by the distillation procedure were too long when the by-product sulfur was being processed at the plant, and the water content was required within a short time in order not to hold up production.

Experimental Work

At the outset the operating engineers requested that a method be developed which would be simple, accurate, and reliable, and have the result available within 10 minutes or less. If such a method could be developed, plant operation and production could proceed normally and without delay.

To meet these requirements a number of procedures were studied and some preliminary work was performed. Some

of the procedures considered were as follows:

1. An attempt was made to measure the time required for a rod to sink into the sulfur paste a given distance. A modification of this procedure was also tried which consisted in measuring the depth to which a rod would sink into the sulfur paste. These procedures did not prove practical, since the reading was affected by the amount of entrapped air, which varied from batch to batch as prepared at the plant.

2. Another procedure tried was to add a known amount of standard acid or alkaline solution and determine the change in concentration caused by dilution with the water present in the sulfur cake. This procedure gave uncertain results and appeared to have too many difficulties to develop further.

3. The next procedure attempted was similar to No. 2, except that the change in concentration was determined by measuring the refractive index. This likewise proved impractical.

4. Attempts were made to separate the water present in the sulfur paste by centrifuging, with and without addition of other substances to aid in such separation. Further work showed that the addition of a small quantity of carbon disulfude in sulfur paste gave a fair separation, but the results obtained were erratic. Further work showed that this procedure was impractical and was abandoned.

5. The determination of the density of the sulfur paste was tried. The density method was given further consideration because it showed from the start that results could be obtained that were within 1 to 2 per cent of the true moisture content when using sulfur from the same batch. However, when sulfur paste from other batches was employed, results were erratic. It was found that the density of sulfur in the by-product material was close to that of sulfur reported in the literature.

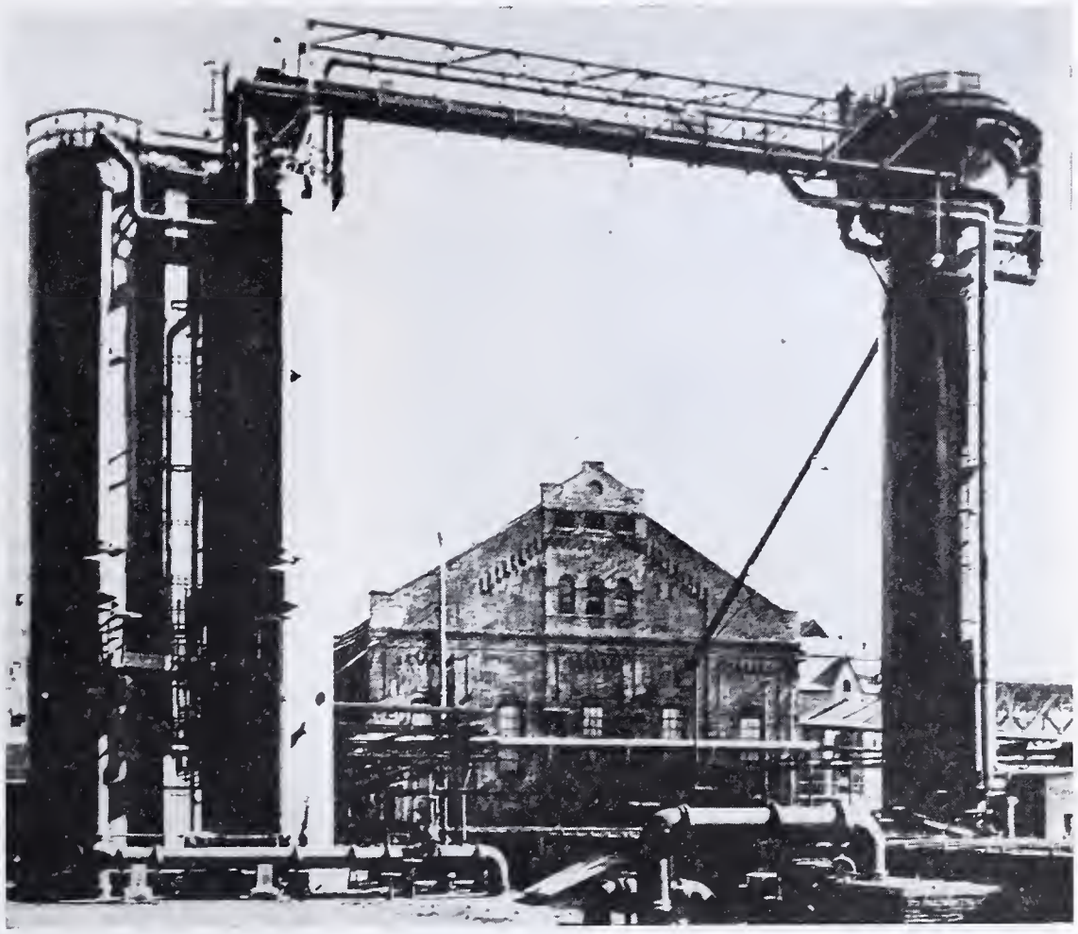

Figure 1. Hydrogen Sulfide Removal System

From previous work carried out in this laboratory on the properties and behavior of by-product sulfur, it was found that various dispersing agents possessed the property of liquefying the sulfur paste, thereby converting it into a creamy solution that could be readily handled. One of the dispersing agents found best for this purpose was Bindarene flour, made by the International Paper Company. The analysis of this Bindarene flour dispersing agent was as follows:

Total solids	96.90
Ash	8.52
Silica and insoluble	0.04
Oxides of iron and alumina	0.11
Calcium oxide	4.60
Magnesium oxide	1.70
Sulfur trioxide	0.36
Sulfur dioxide	4.10
Total sulfur	3.06
pH (of 10% solution)	5.7
Organic matter (100% water ash)	88.39

One procedure employed early in this study was to liquefy the sulfur paste with the Bindarene flour dispersing agent and measure the volume of a known weight of sample. It was observed that the liquefied sulfur paste contained large quantities of small air bubbles which were held in suspension. When these air bubbles were removed, consistent results were obtained.

The procedure finally adopted consisted in general in liquefying the sample of sulfur paste with the dispersing agent, deaerating the sample by suction with constant stirring in a special metal container, and then weighing the sample to determine its moisture content. The detailed procedure is described below.

Apparatus

Figure 2 shows the apparatus partly disassembled. This apparatus consists of:

1. A metal container or deaerating cylinder 31 cm. (12.189 inches) high, 10 cm. (4 inches) in outside diameter, and 0.5 cm. (0.189-inch) in wall thickness.
2. A No. 15 rubber stopper through which the stirrer and paddles pass is used to close the deaerating cylinder, and to support the stirrer and packing gland, and an outlet to the vacuum pump.
3. A proper type of stirrer is required as indicated in Figure 2, where the arrangement and distribution of the paddles are shown, a total of six being used. More paddles are present in the top of the cylinder in order to provide better agitation for deaerating the froth or foam that may be present at the top of the sulfur paste.
4. A heavy-duty, completely enclosed, $^1/_6$ horsepower motor, 1750 r. p. m., is connected to the stirrer by a flexible coupling as shown in the photograph. The motor, stirrer, and cylinder are mounted on a suitable support as indicated. The flexible coupling affords an easy means for removing and inserting the stirrer in the cylinder.
5. A calibrated 500-cc. Florence Pyrex flask.
6. A trap (shown in the right-hand corner) consists of a bottle with rubber stopper in which are two connections, one to the deaerating cylinder, the other to the water suction pump not shown, and a two-way stopcock used to break the suction at the end of the deaeration period.

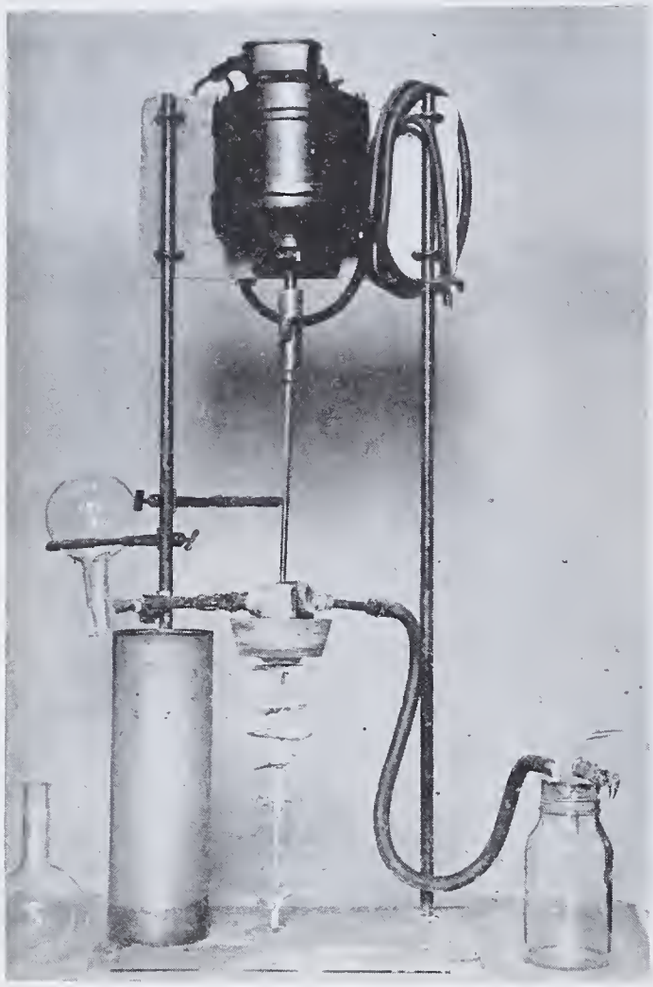

FIGURE 2. DISASSEMBLED APPARATUS

7. Heavy-walled rubber pressure tubing for connections.
8. A suitable torsion or dispersing balance and weights not shown in the photograph are required, and an 800-ml. Pyrex beaker.
9. A 45-cm. (18-inch) wooden spatula has been found most satisfactory for transferring the sulfur paste from the sample can to the metal cylinder. One-gallon enamel milk pails are satisfactory for holding the sample taken from each batch.

Procedure

The procedure as finally adopted consists in placing approximately 800 grams of sulfur paste in the metal cylinder (about half full). This need not be weighed, as a little experience will soon tell how much is right. Next, 4 or 5 grams (a heaping teaspoonful) of Bindarene flour dispersing agent are added. The Bindarene flour is distributed through the paste by mixing with the wooden spatula for about 10 seconds. The metal cylinder with the sulfur paste is then connected to the apparatus, the water suction is started, and the stopcock in the trap is closed. Finally, the motor is started to operate the stirrer. The sulfur paste sample is deaerated under suction with constant stirring for 5 minutes. The motor is then cut off, stopping the stirrer, the stopcock in the trap is opened, and the water suction is turned off. The metal cylinder is disconnected from the apparatus, and the liquefied sulfur paste is poured into a 800-ml. beaker. The calibrated Florence flask is placed on the balance, tared, and 703 grams of the liquefied and deaerated sulfur paste are weighed into the flask. The author has found it simpler to make a special weight weighing exactly 703 grams. The neck of the flask is then washed down with 1 ml. of methyl alcohol to which a small quantity of methyl orange indicator has been added. The water content of the sulfur paste in per cent is then read directly from the calibration on the flask at the level where the top of the meniscus reaches in the neck.

An occasional sample is received with a moisture content below 54 per cent, which is the lowest amount that can be read directly from the flask calibrations. In such a case the procedure is the same, except that after the 703 grams of liquefied and deaerated sulfur paste have been weighed into the calibrated flask, distilled water is added to bring the level up to the 54 per cent mark. The flask is reweighed and the weight of added water is obtained by the difference. This difference divided by 4 and the result subtracted from 54 will give the true moisture content, since, as indicated below, 4 grams of water are equivalent to 1 per cent of moisture in the sulfur paste.

The calibration of the 500-ml. Pyrex Florence flask is carried out as follows:

A Florence flask is selected so that the 54 per cent mark comes up into the lower part of the neck. The 54 per cent mark was found to be the height of 537 grams of distilled water at 25° C. The calibration marks were then determined empirically, using sulfur paste whose moisture content had been determined by the distillation method mentioned above (*8*). Each additional 2 per cent mark, up to and including the 60 per cent mark on the flask, was found to be equivalent to the height of an additional 8.1 grams of distilled water at 25° C.

Table I presents the water content of by-product sulfur paste as determined by the rapid density method, compared to the distillation method (*8*). Some forty-one samples were analyzed. These represent results that have been accumulated over a period of 4 years by at least eight different analysts. The author has on his records over 2200 tests made on sulfur paste by this rapid density method. Examination of these data indicates close agreement between the distillation method and the rapid density method. The average difference between the two methods is 0.1 per cent. The maximum difference, one sample only, is 1.0 per cent. The data further show that results within 0.5 per cent of the true moisture can be obtained by the rapid density method. The rapid density method for moisture in by-product sulfur paste has a number of advantages: No weighing of the original sample is required. It is easily and readily liquefied and deaerated. There is no weighing or measuring to obtain

TABLE I. WATER CONTENT OF BY-PRODUCT SULFUR PASTE

Test No.	Distillation Method	Rapid Density Method	Difference between Methods
	%	%	%
1	57.0	56.5	−0.5
2	57.5	57.0	−0.5
3	57.5	58.0	+0.5
4	58.0	57.5	−0.5
5	56.5	56.5	0.0
6	57.0	56.5	−0.5
7	58.5	58.0	−0.5
8	56.5	56.0	−0.5
9	58.7	58.5	−0.2
10	56.7	56.5	−0.2
11	55.5	55.0	−0.5
12	57.5	58.0	−0.5
13	59.0	59.5	+0.5
14	59.0	58.5	−0.5
15	58.0	58.5	+0.5
16	59.5	59.5	0.0
17	60.5	60.5	0.0
18	57.0	57.5	+0.5
19	57.5	57.5	0.0
20	59.0	59.0	0.0
21	58.5	58.5	0.0
22	59.0	59.0	0.0
23	60.0	59.5	−0.5
24	59.5	59.5	0.0
25	59.0	59.0	0.0
26	57.5	58.5	+1.0
27	57.0	57.0	0.0
28	59.0	59.5	+0.5
29	57.0	57.0	0.0
30	58.0	57.5	−0.5
31	59.0	59.5	+0.5
32	56.0	56.0	0.0
33	56.5	56.5	0.0
34	53.0	53.5	+0.5
35	56.0	55.5	−0.5
36	57.5	57.0	−0.5
37	57.0	56.5	−0.5
38	59.0	59.5	+0.5
39	51.0	50.5	−0.5
40	55.0	54.5	−0.5
41	61.0	61.0	0.0
	Av. 57.6	57.5	−0.1

the final results, but merely a balancing. The moisture content is read directly, no further calculations being required.

After the analyst has acquired experience, a moisture determination can be made within 10 minutes. To speed up the results further, the author has employed two metal containers, so that while one sample is being deaerated, the sample in the other container is being cleaned and made ready for the new determination. Thus by rotating, the time for the determination can be further reduced.

Certain precautions must be taken in the use of this method:

1. Sufficient dispersing agent must be added to liquefy the sample.
2. The entrapped air must be completely removed; otherwise the results will be high. A good test for the completeness of air removal is to tap on the bottom of the beaker or flask containing the liquefied sulfur paste with the finger. If the sound has a ring, the air has not been completely removed. A little experience soon accustoms the analyst to the difference in sound.
3. The distillation method should be used as a periodic check until the analyst has assured himself that the results are correct and that the samples are uniform. An occasional sample has been received from which it was impossible to remove the entrapped air. The samples are generally from sulfur recently made from the sulfur by-product recovery system.

No difficulty has been encountered in the use of the rapid density moisture method, even though used by various members (at least eight) of the laboratory staff. This method is in constant use at this laboratory, especially during the spring when the sulfur paste is being prepared for market.

Acknowledgment

The author wishes to acknowledge the cooperation of members of the laboratory staff, and in particular Theodore R. McCann in carrying out this work.

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Qualitative Test for Methoxy and Other Alkoxy Groups

Compounds Encountered in Pharmacology and Toxicology

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ALTHOUGH numerous modifications of the Zeisel method (*5*) for the quantitative determination of methoxy and other alkoxy groups have been described, and much elaborate apparatus has been designed for the determination, a simple and reliable qualitative test for such groups has hitherto been lacking. Beckmann (*2*) and Feist (*3*) detected methoxy groups in a few compounds by splitting off the methyl radical in such a way as to methylate known compounds, then determining the melting point of the resulting methylated derivative. Such a procedure is slow and inconvenient. Neave and Heilbron (*4*) merely described a very crude qualitative Zeisel procedure, heating compounds to 140° C. with hydriodic acid and passing the evolved methyl or ethyl iodide into silver nitrate, without provision for removing interfering substances which might distill over.

The present paper gives a satisfactory qualitative method for the detection of lower alkoxy groups in soluble compounds of relatively low molecular weight. It is based on the Zeisel method, but requires only a test tube for apparatus, together with a porous plug, impregnated to remove interfering substances. The alkyl iodides produced in positive tests are detected by means of the vermilion color (mercuric iodide) which they give with mercuric nitrate.

Method

Preparation of Porous Plugs. Cut double-thickness cheesecloth (gauze) from a roll 18 inches (45 cm.) wide into strips 2 inches (5 cm.) wide and 18 inches long. Fold each strip once, to get a strip 2 by 9 inches. Place the strips on a sheet of glass. Thoroughly moisten each strip by pipetting 5 ml. of the impregnating solution onto it, taking care that the strips do not curl. Dry the strips on the glass, either by standing overnight or by leaving it in a warm place for 2 or 3 hours.

Impregnating Solution. Dissolve 1 gram of lead acetate in about 10 ml. of water and pour it into 60 ml. of 1 *N* sodium hydroxide in a 100-ml. cylinder. The heavy precipitate which appears soon redissolves. Add a solution of 5.0 grams of sodium thiosulfate in about 10 ml. of water, and 1 ml. of glycerol. Dilute the mixture to 100 ml. with water. The glycerol prevents any of the dried salt mixture from flying off as dust when the strips are rolled before insertion in the test tubes.

Conduct of the Test. Place about 0.1 gram of the substance to be tested (preferably well powdered if a solid), in a 16 by 150 mm. test tube. Carefully pipet 1 ml. of glacial acetic acid and 1 ml. of hydriodic acid (57 per cent, sp. gr. 1.7) onto it. It is desirable, but not essential, to add a small (1 to 2 mm.) granule of unglazed porcelain to promote ebullition. Roll one of the impregnated gauze strips rather loosely into a cylindrical shape and insert it in the mouth of the test tube with a rotary motion. When most of the plug has been inserted into the tube, twist it in the opposite direction to expand it in the bore to a fairly tight fit. There must be no obvious channels by which vapors can bypass the plug. Push the plug down until its top is about 1.5 inches (4 cm.) below the mouth of the test tube. Put a small piece of nonabsorbent cotton (cotton batting) over the plug and press it down into a disk about 2 to 3 mm. thick. Give a strip of filter paper about 2 cm. wide and 10 cm. long a slight longitudinal fold, moisten about a third of its length with a saturated solution of mercuric nitrate in 2 per cent by volume nitric acid, and rest the paper upon the cotton wad.

Place the test tube in a glycerol bath about 4 to 5 cm. deep, maintained at 120° to 130° C. The bath may be heated initially above 130° C., if several tubes are to be immersed in it. Condensed vapors will gradually work up the tube walls into the impregnated plug, which will show a gray discoloration.

With a positive test, a yellow color will spread upward from the bottom edge of the test paper, while changing to a bright

Table I. Detection of Alkoxy Groups

Compound	Alkoxy Groups	Color on Test Paper	Time in Minutes	Result	Explanation
Anisole	1 MeO—	V	7	++++	
Phenetole	1 EtO—	V	5	++++	
Guaiacol	1 MeO—	V	8	++++	
Potassium guaiacol sulfonate	1 MeO—	V	8	++++	S does not interfere in this case
Vanillin	1 MeO—	V	6	++++	
Vanillin acetate	1 MeO—	V	7	++++	
Eugenol	1 MeO—	V	4	++++	
Acetophenetidine	1 EtO—	V	8	++++	
Methyl salicylate	1 MeO—	V + O	4	++++	MeOH hydrolyzed off forms MeI
n-Butyl ether	2 BuO—	Y + O	10	+	B. p. of BuI is 131° C. Ether resists HI
n-Hexyl ether	2 HexO—	Y + light green	10	(—)	B. p. of hexyl iodide is 180° C.
α-Methyl glucoside	1 MeO—	V	2	++++	
Antipyrine	None	Faint buff	10	—	
Piperine	None	Y (very strong)	10	—	Yellow unaccounted for
Dimethoxytetraethylene glycol	2 MeO—	O (strong)	3	+++	
Plasmochin	1 MeO—	Y + O + V	10	+++	
Atebrin dihydrochloride	1 MeO—	O + V	10	+++	
Nupercaine hydrochloride	1 BuO—	O	5	++	B. p. of BuI is 131° C.
Dibutylaminopropyl-*p*-aminobenzoate sulfate (Butyn)	None	Faint gray	10	—	Discolorations probably due to reduction by decomposition products
Azochloramide (Dichloroazodicarbonamidine)	None	Gray	10	—	
Picrolonic acid	None	Y + pale gray	10	—	
Codeine (alkaloid)	1 MeO—	O (strong)	3	+++	
Codeine sulfate	1 MeO—	Y + gray	10	(—)	Discoloration due to S
Brucine (alkaloid)	2 MeO—	V	7	++++	
Quinidine (alkaloid)	1 MeO—	Y + V	10	+++	
Quinidine sulfate	1 MeO—	Y + V	10	+++	No interference by S
Quinine (alkaloid)	1 MeO—	Y + V + O	10	++	Only small spots of V and O
Quinine hydrochloride	1 MeO—	Y + V + gray	10	++	Slight interference by HCl
Quinine sulfate	1 MeO—	Y + gray	10	(—)	S interferes strongly
Narcotine	3 MeO—	V + O	5	++++	
Cotarnine hydrochloride	1 MeO—	Y + O + gray	4	+++	Slight interference by HCl
Thalline sulfate	1 MeO—	Black + gray	10	(—)	S interferes very strongly
Ethylmorphine hydrochloride (Dionine)	1 EtO—	V + O	5	++++	
Cocaine hydrochloride	1 MeO—	O	5	+++	MeOH hydrolyzed off forms MeI
Hydrastine (alkaloid)	2 MeO—	V	3	++++	
Hydrastine hydrochloride	2 MeO—	O	3	+++	No interference by HCl
Morphine sulfate	None	Y (faint)	10	—	No interference by S
Strychnine sulfate	None	Y (strong)	6	—	No interference by S
Atropine sulfate	None	Gray	10	—	S obscures negative reaction

V = vermilion, O = orange, Y = yellow
++++ = strong positive, +++ = moderate positive, ++ = weak positive, + = doubtful positive
(—) = false negative, — = negative

orange or vermilion. Less frequently, the orange or vermilion color will appear rather suddenly over all the moist portion of the test paper. The vermilion may appear as a solid band of color, or as spots against a background of yellow or yellow-orange. A permanent yellow color indicates a negative or a doubtful positive test. The faint yellowish color obtained when the mercuric nitrate solution dries on the test paper is without significance.

The test should be continued until a distinct positive reaction is evident, or until 10 minutes have elapsed. In certain cases, particularly in tests which have been heated for 10 minutes with negative results, a slight gray discoloration may appear on the test paper, probably owing to the action of volatile substances which slightly reduce the mercuric nitrate with the production of finely divided mercury. This discoloration usually fades in an hour or so. Permanent dark gray discolorations due to sulfur compounds are discussed under "Results".

Limits of Sensitivity

The test was applied to 5-, 10-, and 25-mg. portions of vanillin and codeine (alkaloid). The colors were fairly strong with the 25-mg. portions. With vanillin the test was still positive, although weak, with 5 mg. With codeine, the test was still positive (moderate orange color) with 10 mg. but 5 mg. gave only a lemon-yellow color. Accordingly, the limits of sensitivity are about 5 mg. for vanillin and 10 mg. for codeine.

For the detection of methoxy and ethoxy groups in smaller amounts of material (1 to 10 mg.), the A. O. A. C. method (*1*) is undoubtedly superior; sulfur is automatically taken care of, but special equipment and a longer time are required to run the test.

Results

Table I shows that methoxy and ethoxy groups are rather readily detected. With solids, 100 mg. were used; with liquids, 0.10 ml. The "time in minutes" shows when each test was discontinued. In most cases a distinct positive test was obtained in a shorter time. Sulfur often interferes rather seriously with the test, apparently by reduction to volatile mercaptans which are not held back by the impregnated plug. Since the test is invariably satisfactory with free alkaloids, it is desirable to convert most alkaloid sulfates to the free alkaloid before testing. The slight gray discoloration produced by hydrochlorides does not seriously interfere.

Although the butoxy group in nupercaine is readily detected, the test is weak with *n*-butyl ether. Hexyl ether does not react at all. This is undoubtedly due to the high boiling point of the higher iodides, although lower solubility in water and resistance of the simple ethers to the action of hydriodic acid may be a factor.

The "false negative" reactions given in Table I refer to cases in which an alkoxy group is present in the compound, but in which a negative test is obtained, because of either the low volatility of the alkyl iodide or the action of sulfur in obscuring the vermilion color of a positive test.

Acknowledgments

The author wishes to thank Eileen C. Asmuth for technical assistance. Thanks are also extended to Joseph Levine of the U. S. Bureau of Narcotics for samples of certain alkaloids, and to the Union Carbide and Carbon Chemicals Corporation for furnishing other compounds.

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Delivery of Liquids at Low and Constant Rates

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The design of an apparatus for the delivery of small batches of fluid at low and constant rates is presented. Minimum delivery rates are easily and accurately regulated at flows of fractions of cubic centimeters per minute.

Full range control of flow rates is possible. There are no moving parts in the entire unit. Individual parts are obtainable without difficulty or conflict with strategic materials. Only the simplest glass-blowing techniques are involved. Construction and calibration are rapid.

Statics of the apparatus are analyzed to produce an equation useful in design of a unit of proper size for any particular installation desired.

THE apparatus herein presented was suggested by an experiment in which absolutely constant rates of addition of a reagent were required. Moreover, these rates were to be relatively low, in the range of a few cubic centimeters per minute (4 to 5 maximum). Devices which have previously been used with success in other experiments in this laboratory include constant-head tank-meter devices and small pumps of centrifugal or piston design. The main difficulty with the former lies in their lack of adaptability to operation with small batches. Pumps of either design require materials high on priority lists. Centrifugals require auxiliary metering devices, while all piston types, either reciprocating or continuously advancing types, require complicated gearing. In the latter case, full range control is impossible. The present design involves no moving parts, no head tanks, and no meters, and provides full range control.

The design selected relies upon controlled rate of vaporization as the method of regulation. However, the apparatus described is not limited to a pure liquid, since the fluid being vaporized may be completely dissimilar to that whose flow is being regulated. Thus, water may be the material vaporized while a solution may be fed to the final receiver.

[1] Present affiliation, U. S. Naval Reserve.

The maximum capacity of the unit illustrated, with 60 cm. (2 feet) of 32-gage Nichrome wire in the heater, is 4 to 5 cc. per minute, the requirement noted above. Increase of this maximum may be obtained by increase of electrical input (limited in the illustrated unit only by heater design).

Description of Apparatus

As shown in Figure 1, two main units are involved: a still and condenser combination, and a U-tube feeding device. The material to be fed is discharged from the left-hand arm of this latter tube, 11, into any desired receiver. Into the right-hand arm, 9, is fed water, or other suitable liquid, condensed from vapor as delivered by the still. These two liquids are separated by a denser fluid in the bottom of the U-tube, carbon tetrachloride in the illustrated unit. Liquid delivered to the top of the right-hand arm will raise the level of the carbon tetrachloride in the left-hand arm, thereby causing the discharge from the top of a volume of liquid equivalent to the rise in level multiplied by the cross section of the tube.

DETAILS. The still, 1, is an ordinary Dewar flask, so that radiation is minimized and rates of vaporization depend solely upon electrical input. Flask capacity should be that of the amount of water which must be vaporized, plus an amount to keep the heater covered, plus a suitable disengaging area above

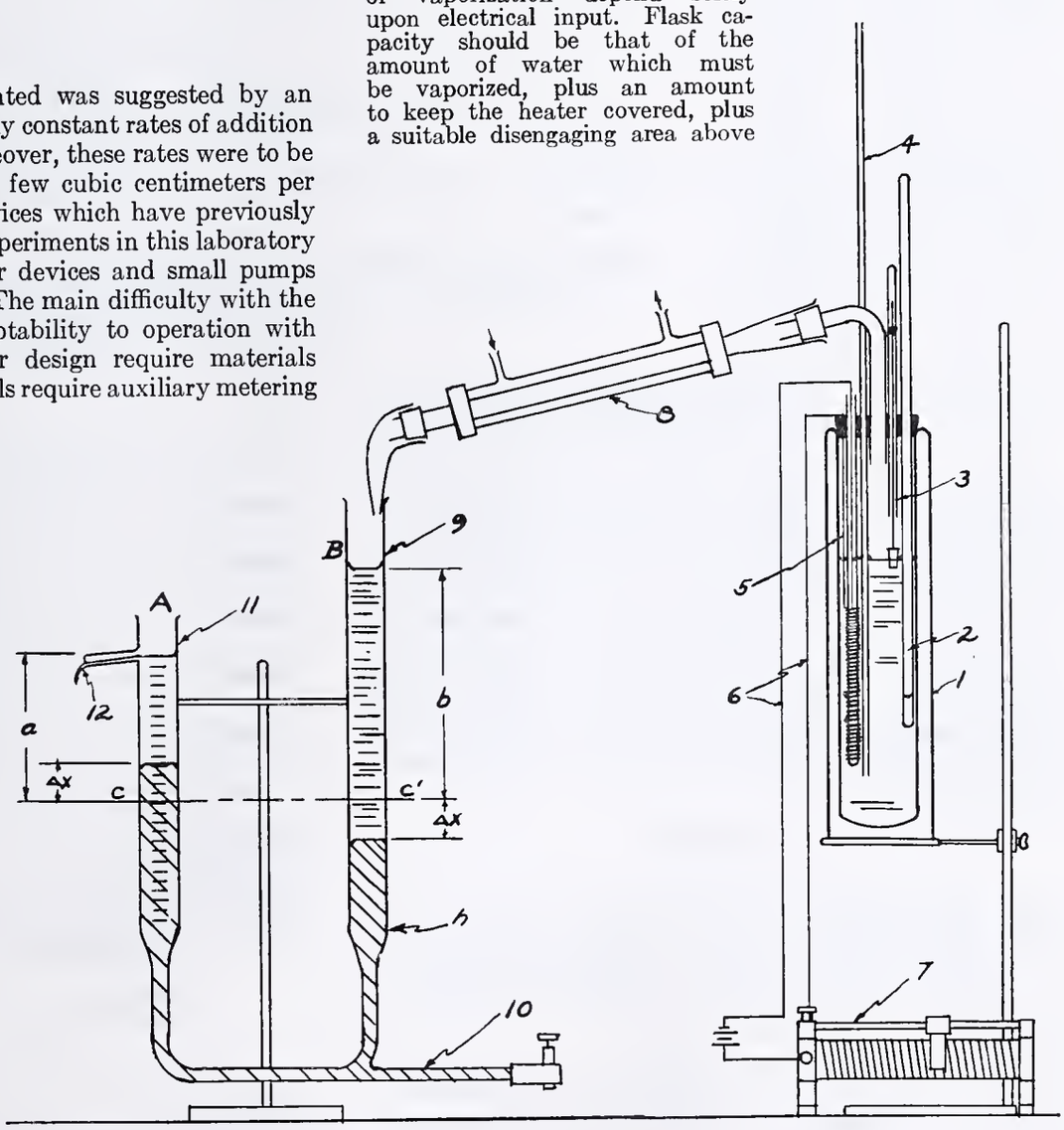

FIGURE 1. DIAGRAM OF ASSEMBLED APPARATUS

1. Insulated flask
2. Thermometer
3. Water level gage
4. Safety tube
5. Heating unit
6. Electric leads
7. Slide-wire resistance
8. Condenser
9. Receiver tube
10. Drain
11. Delivery tube
12. Glass lip

the liquid surface to prevent entrainment. The amount of liquid to be vaporized, which may be calculated from the equations presented at the end of the paper, will vary with density of the three fluids involved, and with the cross sections of the two arms of the U-tube. The still is equipped with any type of electrical submergence heater. Because of the present shortage of equipment, the heater used consisted of a length of fine resistance wire wound on a glass tube, 5, one lead emerging from the inside of the tube. An ordinary knife heater would be suitable, although occupying more space. The level indicator, 3, consists of a piece of cork into which a straight length of wire of moderately heavy gage is fastened. The top of the wire projects above the top of the flask and is enclosed in a glass tube with a sealed top. A long open tube, 4, acts as a pressure safety valve. Figure 1 also indicates a thermometer, 2, extending through a cork into the liquid in the still. Its purpose is only convenience in the prediction of the imminence of boiling.

Control of the heat supplied to the still may be regulated by any combination of resistances or, preferably, by some instrument similar to a Variac transformer which contains a graduated scale. If resistances are used, a scale should be mounted to indicate position of the slides. Ordinary 110-volt alternating current circuits have been found to be a suitable energy source. The small variations in power factor and voltage did not produce a measurable effect in vaporization rates.

Two features of the U-tube are, first, the drain, 10, at the bottom of the U to facilitate recharging and cleaning, and, second, the lip and wick combination, 12, on the discharge from the left-hand arm. The lip serves to direct the flow into the receiver. The wick lies on the bottom of the outlet tube and eliminates fluctuations in flow by preventing periodic accumulation and discharge which would otherwise be occasioned by surface tension.

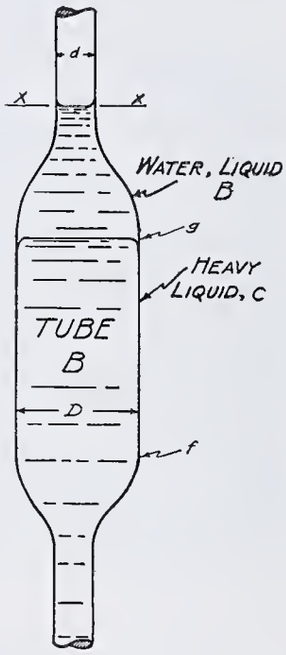

FIGURE 2. SPECIAL FORM TUBE B

As shown in Figure 2, it is possible to vary the cross sections of the two arms of the U-tube. Such variation will result in a marked change in the ratio of volume of the condensate delivered from the still to that of liquid discharged from tube 11. The desirability of such variation can readily be determined by consideration of the discharge equations presented below. All interfaces must travel at all times in a uniform cross section to provide uniform discharge. Thus, in Figure 2, water is shown, prior to operation, standing on top of the heavy liquid to position x, this position to be located in the constricted but uniform top portion of the tube with the heavy liquid interface at g.

Calibration

A few determinations of discharge rate *vs.* rheostat (or Variac) position will serve as a calibration. With a uniformly wound series resistance the calibration plot may be made a straight line if the reciprocal of discharge is plotted *vs.* linear position on the rheostat. Calibration of the instrument illustrated is shown in Figure 3.

Reproducibility of discharge rate at the same resistance reading is excellent, as Table I indicates. Prior to each determination of discharge rate, the contact of the rheostat was moved from and returned to the given setting.

Operation

The U-tube is filled with a heavy liquid and the left-hand side charged with the material to be fed. Since the first few seconds of operation usually give irregular results, this last fluid should be charged in sufficient quantity to bring the level nearly, but not exactly, to the point of discharge. The short time thereby allowed after vaporization commences in the still before fluid flows from the left arm of the U-tube will be sufficient to prevent these initial irregularities from influencing the otherwise constant delivery rate.

The still is also charged with the requisite amount of fluid, presumably water, and current is passed to the heater. As the temperature of the fluid in the still nears the boiling point, current is regulated to the point which, by previous calibration, is shown to correspond to the desired delivery rate.

TABLE I. REPRODUCIBILITY OF DISCHARGE RATE

Slider Setting	Volume Collected	Time Required	Volume Rate	Maximum Variation
	Cc.	*Sec.*	*Cc./min.*	%
(1) 0	5.0	420.3	0.714	
0	5.0	417.0	0.720	0.8
0	5.0	417.9	0.718	
(2) 5	5.0	335.8	0.895	1.1
5	5.0	339.7	0.885	
(3) 10	5.0	250.0	1.200	
10	5.0	250.0	1.200	0.8
10	5.0	248.0	1.210	
(4) 14	10.0	308.5	1.945	
14	10.0	308.5	1.945	0.4
14	10.0	307.5	1.953	

Equation for Volume Rate of Discharge

An equation, derived below, relates the dimensions of the apparatus, properties of fluids involved, and the rate of feed at B, to the desired delivery rate at A (see Figure 1). Limitations placed on the equations are:

1. The diameter of the main body of tube B equals the diameter of tube A.
2. The top interface of the heavy liquid on side B (see Figure 1) never goes below the point of constriction h.
3. When the special design of tube B (Figure 2) is used, the liquid interface is never below point of constriction f nor above that at g.

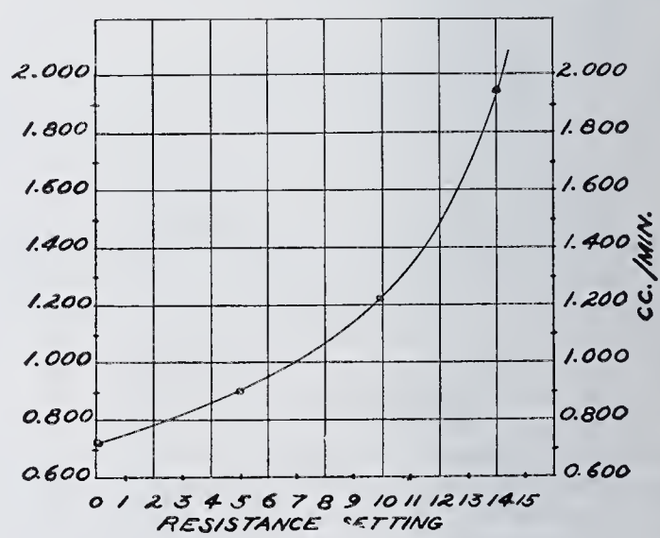

FIGURE 3. CALIBRATION CURVE
Rate of discharge *vs.* resistance position

Limitations 2 and 3 must be maintained for constant delivery rates. Limitation 1 is not required but is convenient in obtaining a simple equation.

If the surface of the heavy liquid moves down a distance ΔX in side B, the general equation for static displacement, as calculated by hydrostatics, is

$$(b + \Delta X)\rho_B = (a - \Delta X)\rho_A + 2\rho_C \Delta X$$

or

$$b = \frac{a\rho_A + \Delta X(2\rho_C - \rho_A - \rho_B)}{\rho_B} \quad (1)$$

b, a, and ΔX are dimensions shown in Figure 1, ρ_A and ρ_B are the densities of the liquids in sides A and B, and ρ_C is the density of heavy liquid.

Let L_1 be the volume rate at which light liquid is introduced at B and let tube B be designed as shown in Figure 2. Then, since ΔX is the displacement of the heavy liquid at C, the volume of light liquid entering tube B is $A\Delta X$, where A is the cross section of tube B where its diameter is D.

Of the liquid charged, $L_1 - A\Delta X$ remains to increase head b and this increase, Δb, may be written

$$\Delta b = \frac{L_1 - A\Delta X}{a}$$

where a is the cross section of tube B where diameter is d.

From Equation 1

$$\Delta b = \left(\frac{2\rho_C - \rho_A - \rho_B}{\rho_B}\right) \Delta X$$

$$\therefore\ L_1 = \left(\frac{2\rho_C - \rho_A - \rho_B}{\rho_B}\right) a\Delta X + A\Delta X$$

and

$$A\Delta X = L_2, \text{ since } D_A = D_B$$

Dividing L_1 by L_2

$$L_1 = L_2 \frac{(2\rho_C - \rho_A - \rho_B)a + A\rho_B}{\rho_B A}$$

or

$$L_1 = L_2 \frac{(2\rho_C - \rho_A - \rho_B)(d/D)^2 + \rho_B}{\rho_B} \quad (2)$$

If the design is that shown in Figure 1, with tubes A and B of equal and uniform diameter, Equation 2 simplifies to

$$L_1 = L_2 \left(\frac{2\rho_C - \rho_A}{\rho_B} - 1\right) + L_2$$

In this case, the capacity of the still must be increased for a given delivery rate, L_2.

Methylene Chloride

In the Extraction and Determination of Vitamin A and Oil in Soupfin Shark Livers

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PEROXIDE-free diethyl ether is generally employed in the extraction of vitamin A–containing oils derived from fish livers. This solvent interferes with the Carr-Price (*1*) and the Rosenthal-Erdelyi (*6*, *7*) colorimetric reactions used to determine the potency of the oil, and must be removed. Furthermore, to prevent an appreciable destruction of the vitamin it has been found necessary to evaporate the solvent in an oxygen-free atmosphere (*3*).

The present investigation shows that if diethyl ether is replaced by methylene chloride in the extraction process, several advantages are realized. The time required for routine colorimetric determinations is considerably reduced, since the solvent containing the oil may be directly added to the chloroform solution of antimony trichloride. The possibilities of vitamin A destruction are reduced by eliminating the necessity of removing the solvent and, therefore, better agreement between duplicate results can be obtained with methylene chloride than with ether extraction. In addition, "refrigeration grade" methylene chloride may be used without further purification.

Experimental

The potency of pooled oils and of oils obtained from individual soupfin shark liver samples was determined by the Carr-Price reaction, its Rosenthal-Erdelyi modification, and ultraviolet absorption. In each case the dilution principle advocated by Norris and Church (*5*) was employed. The color intensity of the Carr-Price reaction was measured at 620 mμ with a Coleman 10-S spectrophotometer (5 mμ slit width), while that of the Rosenthal-Erdelyi reaction was evaluated with a Klett-Summerson photoelectric colorimeter equipped with a green (No. 54) filter. The ultraviolet absorption was determined at 328 mμ with a Beckman quartz prism spectrophotometer, using isopropanol as the solvent.

Each instrument was standardized against the same natural vitamin A ester concentrate. The potency of the concentrate was established spectrographically by Distillation Products, Inc., and was confirmed in this laboratory using the Beckman spectrophotometer. The Beckman and the Coleman spectrophotometers were calibrated with potassium chromate solutions as recommended by Wilkie (*10*).

METHYLENE CHLORIDE AND CHLOROFORM IN THE CARR-PRICE AND ROSENTHAL-ERDELYI REACTIONS. A sample of extracted soupfin shark liver oil (about 0.25 gram) was weighed in a tared 25-ml. volumetric flask. The experimental sample was dissolved in methylene chloride and the control in chloroform. Both samples were further diluted as required and were treated with a chloroform solution of antimony trichloride prepared according to the method of Koehn and Sherman (*4*). The resulting concentration of methylene chloride in the experimental series

TABLE I. COMPARISON OF CHLOROFORM AND CHLOROFORM–METHYLENE CHLORIDE AS SOLVENTS IN THE COLORIMETRIC ASSAY OF VITAMIN A

Reaction	Oil Pool	Colorimetric Determinations: Chloroform	Colorimetric Determinations: Chloroform–methylene chloride
		I. U./g.	*I. U./g.*
Rosenthal-Erdelyi	3	42,900	42,500
	4	386,000	388,000
	x	5,350	5,400
Carr-Price	3	41,800	39,300
	4	370,000	369,000
	5	166,000	166,100
	x	6,100	6,200

amounted to 10 per cent by volume. The same conversion factor was used to calculate the potency of both series shown in Table I.

Methylene Chloride Extraction of Oil and Vitamin A. Methylene chloride was compared with ether in the extraction of oil and vitamin A from shark livers. Liver samples previously frozen in dry ice were homogenized in a Waring Blendor and 2- to 3-gram aliquots were extracted by the short method of Stansby and Lemon (*8*). A 5- or 10-ml. aliquot of the supernatant solution was evaporated to dryness in a tared 50-ml. beaker and the percentage of oil in the liver was calculated from Formula 3.

When methylene chloride was used as the extracting agent, a 1-ml. aliquot of the extract was diluted with chloroform for the colorimetric procedures, or with isopropanol for direct absorption. Ether, when used as the extractant, was removed from the aliquot by immersing the flask in a 40° to 50° C. water bath and blowing a stream of an inert gas (methane) over the solution. The ether-free oil was then diluted to 25 ml. with the appropriate solvent and the weight of oil in each aliquot was calculated from Formula 2. Typical results are shown in Table II.

Calculation of Results. [The procedure of relative aliquots for the simultaneous evaluation of oil and vitamin A in a determination is that used in the Technical Fisheries Laboratory, U. S. Department of Interior, Seattle, Wash. (*2*).]

If the aliquot method outlined above is used, one must bear in mind that the volumes of solvent and oil are nearly additive. The volume of solvent in any given aliquot is then calculated from the approximate formula:

$$V_s = V_a - \frac{W_0}{D} \tag{1}$$

where V_s is the volume of solvent, V_a is the aliquot volume, W_0 is the weight of oil, and D is the average density of the oil.

Using the value for V_s as determined in Formula 1, the oil extracted by 100 ml. of solvent may be found from:

$$\frac{W_0 \times 100}{V_s} \tag{2}$$

and the percentage of oil in the original sample becomes:

$$\frac{W_0 \times 100}{V_s} \times \frac{100}{W_L} \tag{3}$$

where W_L is the weight of the liver sample.

The percentage of oil determined and calculated in this manner was in agreement with values obtained by Soxhlet extraction, and vitamin A potencies were in agreement with determinations made on weighed samples of the extracted oil.

Discussion

Wokes and Willimott (*11*) studied the antimony trichloride–vitamin A reaction in solvents other than chloroform. They dissolved not only the oil, but also the antimony trichloride in these solvents. In all cases the sensitivity of the reaction was diminished, making the substitution inadvisable for many investigations. It was found in this laboratory that antimony trichloride was more readily soluble in methylene chloride than in chloroform. This advantage was offset by the fact that the resulting color intensity was 30 per cent less; however, partial substitution of methylene chloride for chloroform had no effect on the sensitivity of the reaction, as shown in Table I.

According to the results shown in Table II, methylene chloride is as efficient as ether in the extraction of oil and vitamin A. Although no benefit is gained in the subsequent ultraviolet method of assay, a distinct advantage is realized in colorimetric procedures. Moreover, better agreement can be obtained between duplicate determinations when methylene chloride is used in place of ether. The essential validity of this substitution is indicated in the agreement between the results obtained by the physical and chemical methods of analysis.

Attempts to use methylene chloride to extract the unsaponifiable fraction proved unsatisfactory, since persistent emulsions were encountered. Oils of potency less than 10,000 I. U. per gram are seldom received in this laboratory and, therefore, saponification is required in but very few cases.

Even though methylene chloride is less volatile than diethyl ether, great care in handling this solvent is required to prevent loss by evaporation. Moreover, because of the toxicity of this solvent all operations should be conducted in a well-ventilated place. Attempts are being made by others in this laboratory to find solvents which will reduce these disadvantages.

Confirmation of the value of methylene chloride in extracting oils containing vitamins is afforded in the proposed method of the U. S. Pharmacopoeia (*9*) for the assay of capsules of halibut liver oil.

Table II. Comparison of Ether and Methylene Chloride in the Extraction of Oil and Vitamin A

Sample	Oil in Liver: Ether	Oil in Liver: Methylene chloride	Assay Method	Vitamin A[a]: Ether	Vitamin A[a]: Methylene chloride
		%		*I. U./g.*	*I. U./g.*
2	56.0	55.2			
			C-P[b]	...	...
			R-E[c]	127,400	134,500
			U-V[d]	132,000	134,800
4	61.1	61.5			
			C-P	112,600	106,000
			R-E	115,200	113,000
			U-V	109,800	108,200
5	60.3	59.7			
			C-P	104,000	104,000
			R-E	104,000	101,000
			U-V	105,500	104,700
118	79.6	79.3			
			C-P	234,000	236,000
			R-E	232,000	233,000
			U-V	...	...
120	68.2	68.5			
			C-P	27,000	27,200
			R-E	27,000	26,900
			U-V	...	...
122	70.4	70.6			
			C-P	32,950	33,100
			R-E	33,400	33,000
			U-V	...	...
126	53.0	53.3			
			C-P	184,700	185,000
			R-E	183,500	183,000
			U-V	...	...

[a] Assays for a given sample were run on aliquots of same extract.
[b] Carr-Price.
[c] Rosenthal-Erdelyi.
[d] Ultraviolet absorption.

Summary

Methylene chloride is comparable to ether for the extraction of oil in soupfin shark livers, but not for the separation of the unsaponifiable fraction. Vitamin A may be determined directly on an aliquot of the methylene chloride extract. Through the use of methylene chloride it is possible further to simplify an already rapid routine method for the simultaneous determination of oil and vitamin A in these livers.

Acknowledgment

The authors wish to express their gratitude to J. Murray Luck for the keen interest he has shown and the helpful suggestions he has offered while supervising this project. They are indebted to Distillation Products, Inc., for the vitamin A ester concentrate, to the Dow Chemical Company for the methylene chloride (refrigeration grade) used in this work,

and to C. R. Noller of the Department of Chemistry for indicating the possible use of methylene chloride for the work presented in this paper.

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This investigation is incidental to a survey on the vitamin A potency of soupfin shark livers. The survey is part of a program investigating the entire California soupfin shark fishery and is conducted by the California Division of Fish and Game.

Rapid Determination of Sodium Chloride in the Presence of Protein

Application to Salt-Cured Food Products

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Sodium chloride in protein-containing material and solutions is determined by direct titration with 0.0856 *N* silver nitrate, using dichlorofluorescein as an adsorption indicator (40 to 100 mg. of sodium chloride in a volume of 400 ml.). A 0.2 *N* sodium acetate–acetic acid buffer of pH 4.5 is added to the solution or minced sample and allowed to stand or boiled and cooled. The salt is completely extracted from the sample and it is shown that silver is not adsorbed by the protein present at this hydrogen-ion concentration. Results obtained agree closely with the common Volhard method of digestion with nitric acid in the presence of silver nitrate.

PROTEIN interferes in all the usual methods for the determination of chloride, and must be removed by some wet- or dry-ashing procedure. This requires considerable time and inconvenience when large numbers of routine determinations are to be carried out. Attempts to remove protein by various precipitants have not been satisfactory (*2*).

Recently, adsorption indicators have come into common use for the direct titration of halide ions, and in relatively simple solutions the method is very much more rapid and equally as accurate as the common Volhard procedure in which the chloride is precipitated by addition of silver nitrate, and the excess silver ion titrated with thiocyanate (*1, 3, 12*).

Dichlorofluorescein seems to be the most suitable adsorption indicator for the chloride ion in dilute solution (*6, 7, 9, 10*). Here also, low results are obtained in the presence of protein, apparently because of an adsorption of silver on the protein (*6, 11*).

In fish muscle the principal proteins have isoelectric points at about pH 5 and 6 (*8*), and it was reasoned that this adsorption should be at a minimum, and negligible in amount, at a hydrogen-ion concentration near the isoelectric point of the protein micelles. Muscle suspensions and extracts were titrated in sodium acetate and phosphate buffer solutions over the range pH 3 to 9. Theoretical results were obtained between about pH 4 and 4.8 in the presence of protein, while in pure salt solution the indicator was satisfactory between pH 4 and 7. Results have been checked on salt fish, canned fish, fish meals, bacon, etc., and on pickles and brines. The optimum pH range was from 4.3 to 4.8 in each case, and the method as finally developed was found to be entirely satisfactory from an analytical standpoint. In the author's experience, the use of organic solvents as sometimes recommended did not result in any improvement over the above method.

Reagents

Dichlorofluorescein indicator, 0.1 per cent in 50 per cent ethanol.

Acetate buffer, equal volumes of 0.2 *N* sodium acetate and 0.2 *N* acetic acid, adjusted to pH 4.5 (±0.2) if necessary.

Silver nitrate, 14.52 grams in 1000 ml. of solution standardized against pure dry sodium chloride, 1 ml. = 5 mg. of NaCl.

Procedure

Pickles and Solutions. To an aliquot of solution containing about 40 to 100 mg. of sodium chloride, add approximately 20 ml. of acetate buffer solution, and dilute to about 400 ml. with distilled water. Add 10 drops of indicator and titrate with the silver nitrate solution, while slowly swirling the flask, until a pinkish red coloration appears throughout the solution. Titration must be fairly rapid, since the adsorption of the dye considerably increases the light-sensitivity of the colloidal silver chloride, thereby rapidly increasing the rate of aging and decreasing the sharpness of the end point. If the pickle contains much protein, a much sharper end point will be obtained if the solution is boiled for a minute or more after addition of the buffer and cooled to 20° C. before dilution and titration.

Salt Fish and Other Cured Food Products. A well-comminuted sample is mixed with 25 ml. of buffer, and diluted to about 75 ml. with water. The mixture is boiled for a few minutes, and cooled. The whole may then be diluted to 400 ml. and titrated as before, or the solution may be made to 100 ml. and an aliquot of the supernatant liquid used for the titra-

tion. The weight of sample used will depend on the salt content and degree of subdivision of the sample. If it is desirable to use a large sample for the sake of uniformity in sampling, the titration of an aliquot containing 40 to 100 mg. is the preferable procedure. Either fresh or dried samples may be used, so that moisture and salt analyses are often conveniently made on the same sample. If a 0.5-gram sample is used, as is convenient with salt fish, the percentage salt content is read directly on the buret, 1 ml. being equivalent to 1 per cent of sodium chloride in the original material.

Table I. Recovery of Added Sodium Chloride

Sample	NaCl Added	NaCl Found	Recovery of Added NaCl
	Mg.	*Mg.*	%
Fresh cod, 0.5 gram	0	0.5[a]	...
	25	25.3	101
	50	51.0	101
Light salt cod, 0.5 gram	0	60.0	...
	25	85.2	101
	50	109.5	99
	75	135.0	100
Hard salt cod, 0.5 gram	0	97.5	...
	25	122.0	98
	75	170.3	97

[a] Volhard method.

Results and Discussion

A series of titrations was conducted on pure sodium chloride solutions and on extracts of salt fish and of fish meal with sodium chloride added, over a range of pH 3 to 8 in sodium acetate and phosphate buffers. The results are shown in Figure 1.

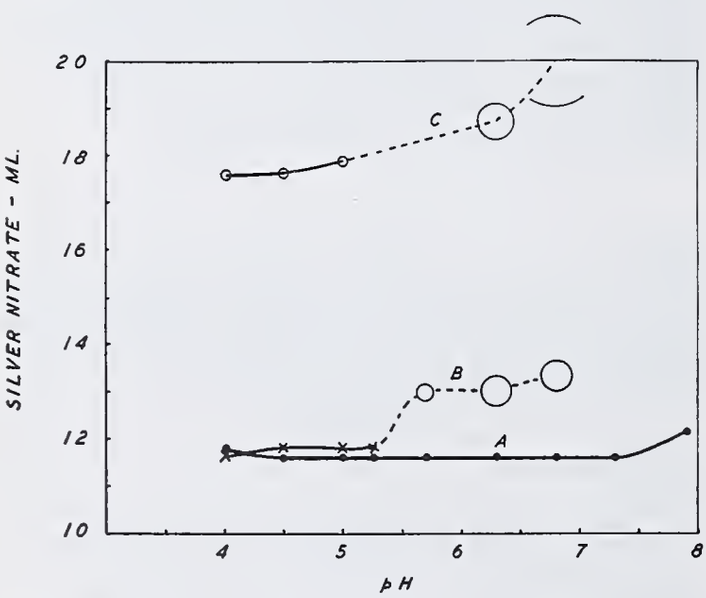

Figure 1. Influence of pH on Titration End Point

1 ml. of $AgNO_3$ soln. = 5 mg. of NaCl

A. Standard solution of sodium chloride. Titer = 11.60 ml.
B. Standard solution of sodium chloride + 1 gram of fresh cod muscle. Titer = 11.80 ml.
C. Standard solution of sodium chloride + extract of 1 gram of fish meal. Titer = 17.60 ml.
Dotted lines show indefinite end points—approximate limits shown.

At acidities below pH 4 the indicator showed no color change, and above pH 7.5 the change became indefinite, an orange-yellow or yellow color being obtained. In the presence of protein the end point becomes indefinite above approximately pH 6, at an acidity just below the point where an increased adsorption of silver occurs. The amount of silver and dye ions adsorbed by the protein increases as the solution is made more alkaline, and thus no definite end point is obtained in this range.

It is indeed shown that between pH 4 and 5 a range probably slightly more acid than the isoelectric point of the known proteins present, little or no adsorption of silver or of chloride occurs. In solutions containing considerable soluble protein, such as those from fish meal where the salt content is low and consequently a high dilution cannot be used, a pH of 4.2 to 4.8 gives results agreeing with the Volhard method. A buffer of pH 4.5 was accordingly selected for routine use.

This indicates that cod and haddock muscle has an apparent isoelectric point near pH 4.5. Other work on the salting of cod muscle (*4*) has also shown an apparent isoelectric point between pH 4.0 and 4.5. Further study of the proteins present is needed, although the isoelectric point does not necessarily coincide with the point of minimal acid and base-binding capacity (*5*).

Recoveries of added sodium chloride are shown in Table I and agreement of results with those of the standard Volhard method in Table II, on samples of salt cod, fish meal, and bacon. The fish meal samples were analyzed by boiling a suspension of 5 grams of meal in 100 ml. of buffer and water, then adding 50 mg. of sodium chloride (standard solution), to a 20-ml. aliquot of the suspension. Otherwise the salt content was too low, and insufficient colloidal silver chloride was formed to allow easy observation of the end point.

Table II. Comparison of Adsorption Indicator and Volhard Methods

Sample	Sodium Chloride Found: Adsorption indicator	Sodium Chloride Found: Volhard
	%	%
Hard salt cod, 0.5 gram	19.8 (±0.2)[a]	19.9 (±0.3)[a]
Light salt cod, 0.5 gram	11.3 (±0.2)[a]	11.2 (±0.2)[a]
Bacon, 5 grams	1.30 (±0.03)[a]	1.31 (±0.03)[a]
Fish meal, cod	3.00, 2.95	2.93, 2.91
Fish meal, herring	0.75, 0.70	0.72, 0.73

[a] Average of 4 determinations.

Recovery of added salt is very satisfactory, and the results agree with the standard method.

Complete extraction of salt from a minced sample by the buffer solution was obtained. Boiling for 2 to 5 minutes, followed by cooling to room temperature, gave the same results as extracting for approximately 3 hours at room temperature, and the end point was considerably more satisfactory. Decreasing the extraction time to less than 3 hours at room temperature resulted in incomplete extraction, as found by comparison with the wet-digestion methods.

The presence of fat or oil in fish meal or meats had no influence on the results. The method has been in continuous use for more than a year in the research and routine analyses of salt fish and other fishery products.

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Spectrophotometric and Biological Assay of Vitamin A in Oils

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IN AN earlier publication (*2*) comparisons were made between the biological and spectrophotometric methods of assay of vitamin A in a series of fish liver oils. Since that publication a new U. S. P. reference cod liver oil for biological assay of vitamin A has been introduced. Spectrophotometric tests on the stability of this reference have been performed and new comparisons between the two methods of assay have been made.

Experimental Procedure

PHYSICAL. The instrument used was a Judd Lewis ultraviolet photometer with the different photometer units, including the source, rigidly mounted to the frame of a medium Bausch & Lomb ultraviolet spectrograph. The light source was a tungsten-steel spark. The density scale of the lower sector of the photometer was calibrated over a range of density values of 0.60 to 1.00 against three different concentrations of potassium chromate solution for each sample tested. Isopropanol was used as a solvent for all the oils. Two weighings and two dilutions of each weighing were made for each sample. Match points were read from the image (magnification factor 6) of the plate projected on a white screen by a modified Bausch & Lomb projector.

The assays of the oils of lower potency were made on the unsaponifiable fractions and in some cases also on the whole oils. Two methods of saponification (*2*) were used for each oil.

BIOLOGICAL. The U. S. P. XI procedure was followed in all cases and the oils were handled in the manner previously reported (*2*).

TABLE I. U. S. P. REFERENCE COD LIVER OIL 2

No.	$E^{1\%}_{1\text{ cm.}}$ (Unsaponifiable Fraction)	No.	$E^{1\%}_{1\text{ cm.}}$ (Whole)
1	0.78	1	0.86
2	0.83	2	0.86
3	0.75	3	0.86
4	0.75	4	0.86
5	0.81	5	0.88
6	0.69	6	0.85
7	0.70	7	0.85
8	0.70	8	0.82
9	0.71	9	0.79
10	0.69	10	0.86
11	0.73	11	0.85
12	0.74		$E^{1\%}_{\text{cm.}}$ = 0.85 (coefficient of variation 2.86)
13	0.77		
14	0.76		

$E^{1\%}_{1\text{ cm.}}$ = 0.74 (coefficient of variation 5.95)
Conversion factor (unsaponifiable fraction), 2300
Conversion factor (whole), 2000
Based on value of 1700 U. S. P. per gram.

TABLE II. STABILITY OF U. S. P. REFERENCE COD LIVER OIL 2

Bottle No.	Date	$E^{1\%}_{1\text{ cm.}}$	No. of Measurements
1	11/1/40	0.79 (unsaponifiable fraction)	1
		0.86 (whole)	1
	12/1/40	0.79 (unsaponifiable fraction)	1
		0.88 (whole)	1
2	4/4/41	0.77 (unsaponifiable fraction)	3
		0.87 (whole)	3
	4/6/42	0.68 (unsaponifiable fraction)	3
		0.82 (whole)	3
3	4/7/41	0.72 (unsaponifiable fraction)	1
		0.84 (whole)	1
	11/7/41	0.64 (unsaponifiable fraction)	1
		0.86 (whole)	1
	4/15/42	0.60 (unsaponifiable fraction)	3
		0.77 (whole)	3

Results

In Table I are listed values obtained by spectrophotometric assay of U. S. P. reference oil 2. These measurements were made over a period of 2 years on freshly opened samples of the reference oil and each sample was tested within the date labeled as safe. No significant variation of measurement was obtained among the samples tested.

Table II includes data on the stability of standard reference 2 as indicated by assays carried out on three bottles. The assays were made when the bottles were first opened and also after intervals varying from one month to a year. After each measurement the bottles were flushed with carbon dioxide, stoppered, and stored in the refrigerator. A loss in vitamin A potency after several months was indicated.

TABLE III. FISH LIVER OILS

Source of Vitamin	No. of Samples	Potency Range, Biological Units	Average Conversion Factors	Coefficient of Variation[a]
Cod liver oil (unsaponifiable fraction)	32	1,000–4,850	2170	6.54
Cod liver oil (whole)	12	1,000–4,850	2070	9.32
		Higher Potency Oils		
Capsules	4	1,330–9,700	1900	3.23
Tablets	8	2,070–11,800	2030	7.58
Shark liver oil	12	4,500–98,000	1910	6.54
Tuna liver oil	9	12,500–85,000	1910	7.27
Halibut liver oil	10	38,000–201,000	1960	8.06
Vitamin A ester concentrates	7	202,000–520,000	1970	4.82
Vitamin A concentrates	7	205,000–1,200,000	1850	6.00
Miscellaneous	4	7,800–9,200	2050	3.93

Weighted average conversion factor for higher potency oils, 1940.

[a] Coefficient of variation = $\frac{\text{standard deviation} \times 100}{\text{average conversion factor}}$

Table III lists values obtained with 93 samples of fish liver oils varying from cods of biological potency of 1000 units to concentrates of over 1,000,000 units per gram. These are segregated into groups. The number of samples tested, potency range in biological units, average conversion factors, and coefficients of variation are listed. The conversion factors for the oils of higher potency are in good agreement, but the average factor of 1940 for this group is smaller than that of 2170 for the unsaponifiable fraction of the cods. The 93 samples represent all the samples tested over a definite period, with the exception of two multiple vitamin capsules. Absorption curves for these two atypical samples, along with that of a sample giving a typical vitamin A curve, are shown in Figure 1.

Table IV compares the conversion factors of the two reference oils based on the claimed unit contents and also the conversion factors of the test oils which are computed from the biological assay results obtained with the two reference samples.

Discussion of Results

The data in Table IV show that the average conversion factors for the test oils have undergone a significant decrease

Table IV. Conversion Factors

	No. of Measurements	Conversion Factor	Coefficient of Variation
U. S. P. reference 1 (unsaponifiable fraction)	14[a]	2180[b]	2.40
U. S. P. reference 2 (unsaponifiable fraction)	14	2300[c]	5.95
Cod liver oil (unsaponifiable fraction), Series I[d]	22	2700[b]	10.30
Cod liver oil (unsaponifiable fraction), Series II	32	2170[c]	6.54
Higher potency oils, Series I[d]	31	2260[b]	9.60
Higher potency oils, Series II	61	1940[c]	7.01

[a] Measurements made on four freshly opened bottles of U. S. P. reference 1, averages given in (2). 1-gram samples used.
[b] Based on biological value of 3000 U. S. P.
[c] Based on biological value of 1700 U. S. P.
[d] See (2).

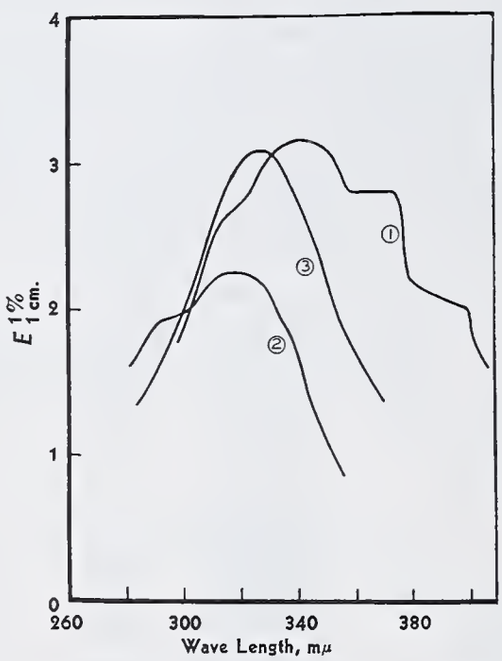

Figure 1. Absorption Curves in Isopropanol Solution

1, 2. Experimental multiple vitamin capsules giving atypical vitamin A absorption curves
3. Experimental multiple vitamin capsule giving a normal vitamin A curve

with the advent of U. S. P. reference cod liver oil 2. Since there has been no change in the spectrophotometric technique, it is probable that this change is associated with the biological test. Such a lowering of the conversion factor could result from differences in the size of the unit of vitamin A in the two reference samples, but such an explanation is not consistent with all the facts. The conversion factors of reference samples 1 and 2 computed on the basis of the claimed unit content are in fair agreement; in fact, that of the former is slightly lower than the latter. It would appear more probable that such a lowering of the conversion factors is due to differences in the stabilities of the two reference oils. A relatively short time elapses between the opening of a fresh bottle of reference and the completion of a spectrophotometric test, whereas there is considerable opportunity for loss of activity to occur before and after the feeding of the diluted reference to the test rats. Thus, if reference 2 were more stable than reference 1, it would produce the same effect in the biological test as would a reference containing larger units.

Spectrophotometric data listed in Table II on reference oil 2 and data previously published (2) on reference oil 1 indicate that neither of these oils as received from the U. S. P. committee has shown any loss in potency; however, spectrophotometric measurements on reference 1 suggest a rapid loss of vitamin A potency in samples which had been opened and allowed to stand in the refrigerator, as evidenced by a drop in the $E^{1\%}_{1\,cm.}$ value of about 14 per cent. Similar measurements on the new reference also suggest a loss of vitamin A potency with time as indicated in Table II, but the deterioration appears to be less rapid than that for reference 1, a drop of 14 per cent occurring after several months rather than weeks.

It would thus seem that improvements could be made on the stability of the reference oil, possibly by the use of antioxidants. It was pointed out by Dyer *et al.* in 1934 (3) that the biological activity of carotene or cod liver oils was affected by the oil which was used as a diluent for feeding. Recently Hickman (5) and Quackenbush (6) have shown that vitamin E possesses antioxidant properties for vitamin A or carotene in vivo as well as in vitro. These studies suggest that some of the variations in biological assays may be due to differences in the content of vitamin E or other antioxidants in different samples. Studies of the problem are now in progress in this laboratory.

A survey of the data listed in Table III indicates a high degree of correlation between the spectrophotometric and biological assays of vitamin A. The average conversion factor of 1940 for the higher potency fish liver oils is lower than the value of 2000 recently proposed as a working value for comparisons between measurements made in different laboratories. This factor is lower than that of 2170 found for the unsaponifiable extract of cods. All these factors are lower than that of 2460 reported by Baxter and Robeson (1) for crystalline vitamin A.

There were only two samples in this survey which did not give comparable results by the spectrophotometric and biological methods. Absorption curves of these samples are given in Figure 1. Curve 1 is the absorption curve of an experimental multiple vitamin capsule, in which the biological assay gave a lower value than did the spectrophotometric assay. The absorption curve is resolved into three distinct "flats" in the regions of 340 mμ, 365 mμ, and 390 mμ and resembles the absorption curve of a mixture of vitamin A and cyclized vitamin A as reported by Embree (4). The interpretation was that the vitamin in the capsule had been partially cyclized and hence a measure of the absorption at 328 mμ did not give a true measure of the vitamin A potency.

The second case was that of another experimental multiple vitamin capsule, the absorption curve of which is plotted as curve 2 in Figure 1. In this case the absorption peak was shifted to 318 mμ; in addition there was a rather high flat at 300 mμ and the ratio of biological assay to $E^{1\%}_{1\,cm.}$ was low. This seems to compare with a case of destruction due to oxidation as reported by Robinson (7). In neither case could the spectrophotometric method give an accurate value of the vitamin A potency of the sample, and the inaccuracy of the assays was indicated by the absorption curves.

Summary

Spectrophotometric measurements on fresh samples of U. S. P. reference sample 2 over a period of 2 years are recorded. The average conversion factor, computed on the basis of claimed content of 1700 U. S. P. per gram, is 2280 for the unsaponifiable fraction and 2000 for the whole oil.

The conversion factors computed from biological and spectrophotometric measurements on 32 cods and 61 oils of higher vitamin A potency show a decrease of from 20 to 14 per cent from those computed when the older standard reference oil 1 was used in biological assays. The new conversion factors are 2170 for the unsaponifiable fraction of the cods and 1940 for the higher potency oils. The suggestion is made that such a decrease in the conversion factors is due to a difference in the stability of the two references.

There was good agreement between the results of the biological and spectrophotometric tests of all but two samples. The absorption curves of these were not typical for vitamin A and thus served as an indication that the spectrophotometric method could not give an accurate assay in such cases.

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Laboratory Cooking, Mashing, and Fermentation Procedures

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A procedure is presented for conducting test laboratory fermentations for the production of alcohol from corn, wheat, or rye. The analytical procedures and methods of calculating results are described. The procedure is sufficiently flexible to be used for testing a variety of fermentation conditions or fermentation raw materials.

THERE has been no generally accepted method for laboratory studies of the grain alcohol fermentation process. During the course of numerous studies involving the fermentation of corn, wheat, and rye mashes, it was necessary to develop an accurate procedure yielding reproducible results. The objective was a method which would yield experimental results closely comparable with production data. Thus a standard control by this technique should yield as much alcohol as could be expected in an efficiently operated distillery when the same grains are processed. Furthermore, since it was necessary to evaluate the results obtained when varying one processing condition such as a conversion, cooking conditions, or type of grain, the method had to encompass a high degree of accuracy. As a result of the continued improvement and extensive use of this procedure, the authors are now convinced of the accuracy of the method and feel that the experimental error is less than 1 per cent. Only ordinary laboratory equipment is required.

This paper presents detailed procedures for the preparation of the mash and the analytical methods employed to obtain essential data. Since distillery nomenclature is often confusing, the terms are defined and all calculations are explained and shown by example. Typical laboratory fermentation data are presented. Below is the procedure for preparing 1.5 liters of mash at a concentration of 38 gallons of mash per bushel of grain (gallons of mash per bushel, the total volume in gallons which contains 1 bushel of grain).

Cooking and Conversion Procedures

Pressure Cooking. *Grain Bill*, 92 per cent corn or wheat, 8 per cent distillers' barley malt (Lintner value—minimum 175° dry basis).

Meal Analysis. These grinds (Table I) can be obtained by grinding through the medium screen of the Wiley mill. Other mills may be substituted, provided comparable grinds are obtained.

Moisture. At the time of mashing weigh a 10-gram sample of each of the ground grains employed into a tared dish, dry this sample at 110° C. for 3 hours, and reweigh.

Starch Content. Starch analysis is made by the A. O. A. C diastase–hydrochloric acid method (*1*).

Cooking. Place 883 cc. of tap water in a 2-liter beaker. Clamp the beaker in a water bath (any suitable vessel), so that the beaker is at least half submerged. The water in the beaker should be mechanically agitated with a propeller driven by a variable-speed laboratory motor.

Heat to 100° F. (temperature of water in beaker) and add 2.33 grams of distillers' barley malt. (Fahrenheit temperatures are used in conformity with industrial practice.)

Heat to 130° F. and add 241.2 grams of corn or wheat.

Remove source of heat.

Check pH, taking care to keep the entire procedure as quantitative as possible.

Adjust pH to 5.6 with *N* sulfuric acid.

Heat to 170° F. (at such a rate that this temperature is attained in about 20 minutes), measure, and record the volume by dry inches (the number of inches from the surface of the mash to the top of the container).

Increase the temperature to approximately 200° F. as rapidly as possible and hold at this temperature for 1 hour. However, start timing when the temperature reaches 185° F.

At the end of 1 hour make up the volume to equal that at 170° F.

Autoclave at 22 pounds for 1 hour. (The mash should be mechanically agitated at all times except when checking dry inches and when the cook is autoclaved.)

Table I

Grind	Mesh	Corn, %	Wheat, %	Barley Malt, %
On	12	0.0	0.0	0.0
On	16	0.5	0.0	0.5
On	20	2.0	6.0	1.5
On	30	25.5	55.5	11.0
On	40	32.0	22.0	36.5
On	60	37.5	8.0	26.5
Through	60	12.0	14.5	23.5

Conversion. After removing the cook from the autoclave place it in a water bath and agitate mechanically. It is important to have the temperature of the water bath between 160° and 170° F. at the time the cook is added. Cool the water bath to 145° F. and add the conversion slurry, consisting of 140 cc. of water and 20.95 grams of distillers' barley malt, when the temperature of the cook reaches 152° F. Make the malt slurry 30 minutes before using and heat to 130° F. immediately before adding it to the cook; warm the slurry in a water bath with occasional stirring.

Flash Conversion Method. As soon as the malt slurry is added to the cook, stir until there are no lumps (manual as well as mechanical stirring is frequently required) and then cool as rapidly as possible to 72° F. The conversion pH should be 5.4 to 5.5 (this need not be checked, since the adjustment of the pH for cooking will place the conversion pH in this range).

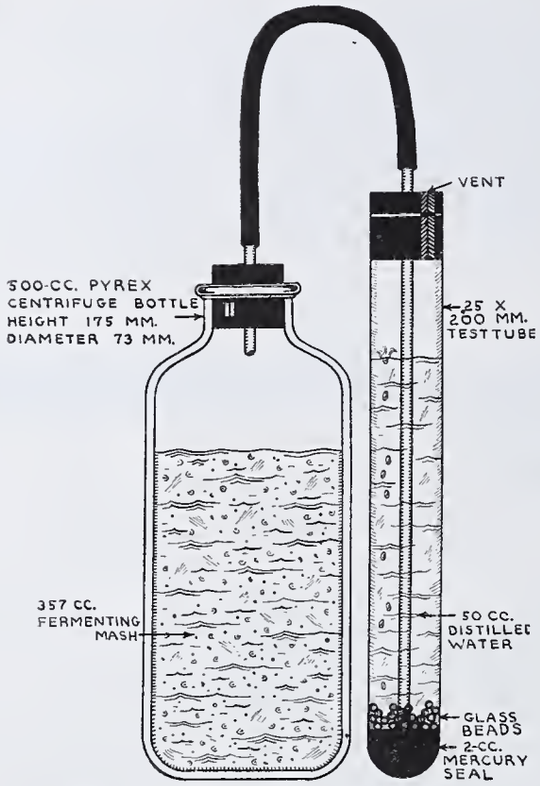

FIGURE 1. LABORATORY FERMENTATION FLASK EQUIPPED WITH CARBON DIOXIDE SCRUBBER

Sixty-Minute Conversion Method. Flash conversion is a superior production method which is gaining favor in the industry and normally results in increased alcohol yields. However, this method occasions more difficulty in the laboratory if efficiency data, based on sugar analysis, are desired (see method B, section on calculation). As an alternate, the 60-minute conversion method (comparable with the old standard plant procedures) may be employed. In this case, the mash is mechanically agitated after addition of the malt slurry for 60 minutes while held at 145° F., then cooled rapidly to 72° F.

INFUSION PROCESS (ATMOSPHERIC MASHING). The procedures described above are used frequently in research and control work in connection with modern grain alcohol plants. However, it is occasionally desirable to employ the infusion process when testing the fermentation of wheat, rye, or malt mashes. These grains do not require pressure-cooking for efficient gelatinization of the starch.

Wheat. Heat 883 cc. of tap water to 100° F. as above, add 241.2 grams of wheat slowly, raise the temperature to 155° F. in 45 minutes, and hold for 60 minutes; then cool to 152° F. and add the malt slurry containing 23.3 grams of malt prepared as described above. Hold the mash another 30 minutes at 145° F. and then cool to 72° F. The mash must be mechanically agitated at all times.

Rye. Heat 883 cc. of tap water to 100° F., add 23.3 grams of malt slowly, agitate the slurry 5 minutes, then add 241.2 grams of rye slowly. Raise the temperature to 130° F. in 30 minutes, hold 15 minutes, raise to 145° F. in 15 minutes, and hold at 145° F. for 60 minutes. At the end of this period cool the mash rapidly to 72° F. The mash is to be mechanically agitated at all times.

Fermentation Conditions

PREPARATION OF YEAST FOR INOCULUM. Transfer one loop of yeast from slant to test tube containing 10 cc. of 16° Balling malt extract. Transfer every 24 hours for 2 days through the same medium, transfer contents of the last tube to 200 cc. of the same medium, incubate for 20 hours at 86° F., and then use for inoculum.

PROCEDURE FOR SETTING FERMENTERS. This is a critical step in the procedure. Failure to observe reasonably aseptic conditions will result in contaminated fermentations and nullify the final data. Lack of observance of quantitative technique has the same result.

Pour cooled converted cook into clean, sterile 2-liter graduated cylinder.

Add 300 cc. of sterile thin stillage (whole residue from distillation of alcohol from fermented mashes. Thin stillage is screened.) Water may be used when stillage is unavailable.

Mix thoroughly with propeller.

Adjust pH to 4.8 with *N* sulfuric acid if necessary.

Make volume up to 1500 cc. with tap water.

Mix well by pouring back and forth from graduate to beaker once or twice.

Check pH; if above 4.85 set back to 4.8 with a few drops of 4 *N* sulfuric acid.

Add 7 cc. of yeast (2 per cent of total mash volume) to each sterile, tared fermenter.

Measure mash into fermenters as follows: Rinse dry sterile 500-cc. graduate with 50 cc. of mash. Measure out 350 cc. of mash and pour into fermenter (Figure 1), allowing to drain until there is only 1 drop every 2 seconds.

Fill three fermenters from each cook as described above. Use a different graduate for each cook. Mix mash in beaker well each time before pouring into cylinder.

Attach trap to fermenter as follows (see Figure 1): Place 2 cc. of mercury, glass beads to a depth of about 1.25 cm. (0.5 inch) and 50 cc. of distilled water in a 25 × 200 mm. test tube. A glass tube extends to the bottom of this trap and is connected with a one-hole rubber stopper in the fermenter by a short piece of rubber tubing. The connection between trap and fermenter should be sterile.

INCUBATION. It has been the practice to incubate mash fermentations in a constant-temperature water bath (Figure 2). Air incubation may be used, but it is believed that baths are more satisfactory.

Incubate at 72° F. for the first 20 hours.

Incubate at 86° F. for the next 30 hours.

Incubate at 90° F. for the final 16 hours.

At least twice during fermentation, at 20 and 50 hours, shake the fermenter thoroughly by swirling until all mash adhering to the bottom of the flask is in suspension.

Fermentation Analysis

The majority of the work in these laboratories has necessitated only initial final analyses. Periodic analyses may be made, but when maximum accuracy is desired only final analyses should be determined. The individual will vary these procedures to fit his own requirements. These again are critical, since work of this type frequently involves slight differences in yield, which are significant and must be so established. The analyses to be made will depend on the data desired, which can be selected after reading the section on calculations.

INITIAL DATA. *Sample.* The initial data are obtained by the analysis of the mash remaining after the fermenters are set. All analyses are run on the supernatant obtained by centrifuging the mash, with the exception of initial total sugar analyses of flash converted mashes, which are treated as described below. The mash is centrifuged at 2000 r. p. m. for 10 minutes. Filtration through coarse paper may be substituted.

a. pH. Take pH using potentiometer and glass electrode.

b. Titratable acidity. Titrate 10 cc. of centrifuged sample to the phenolphthalein end point with 0.1 *N* sodium hydroxide. Record as cc. of 0.1 *N* sodium hydroxide per 10 cc. of sample.

c. Sugar concentration.

1. Maltose. Determine reducing sugar in centrifuged sample of mash by the method of Stiles, Peterson, and Fred (*2*), (20 minutes' oxidation and employ correction factor determined for reagent on maltose).

2. Total sugar, flash converted mashes. As soon as fermenters are set, weigh 100 cc. of excess mash into small Erlenmeyer flask. Adjust pH to 5.4 to 5.5 with a drop of concentrated sodium hydroxide and complete conversion by holding in the water bath to 145° to 150° F. for 1 hour. Shake every 5 minutes, being careful not to splash mash high on the side of the flask. Adjust to initial weight with water and centrifuge sample. Hydrolyze exactly 5-cc. sample with 5 cc. of 1.38 *N* hydrochloric

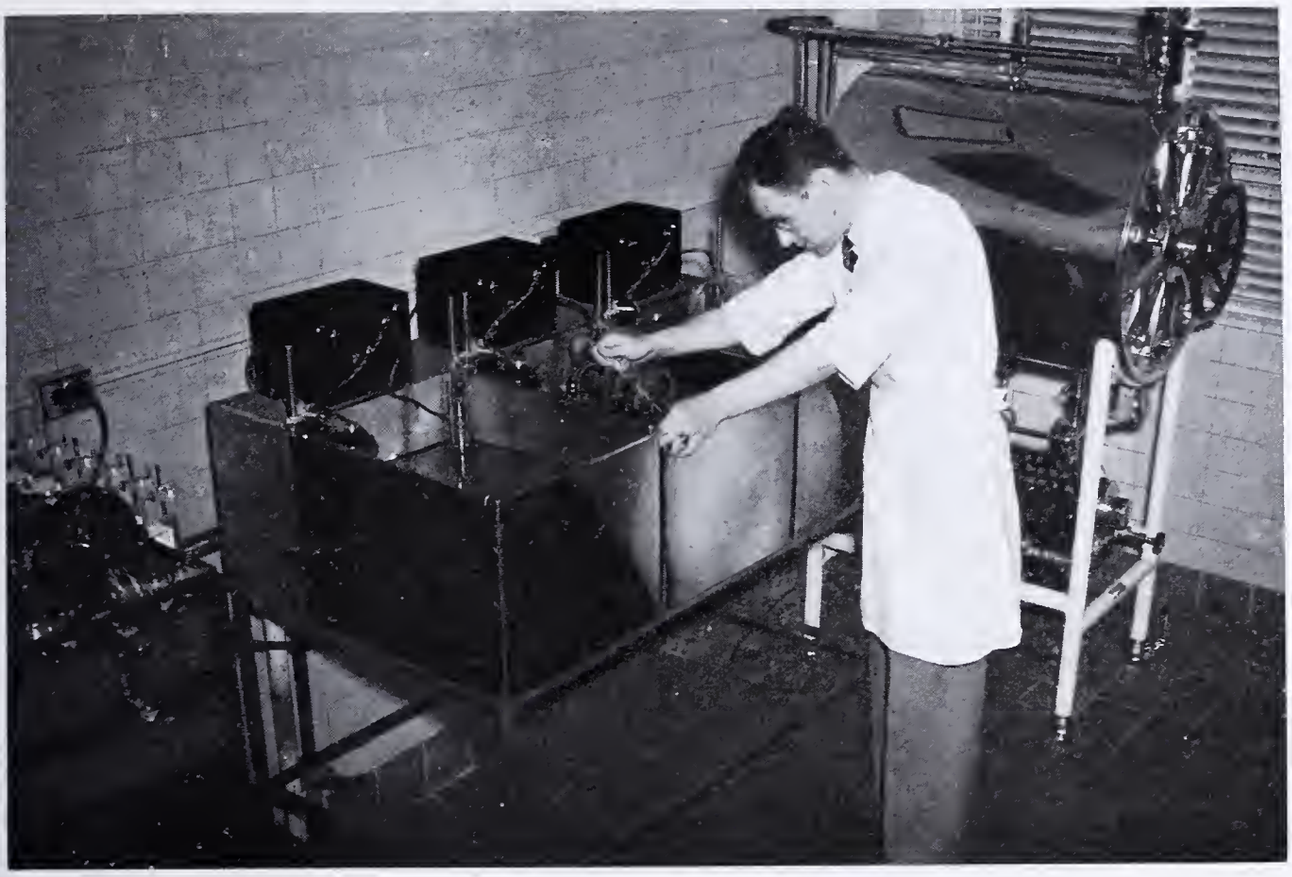

FIGURE 2. LABORATORY WATER BATHS

Equipped with agitators, thermoregulators, heating units, and cooling coils for accurate temperature control

acid by boiling for 2.5 hours, then run sugar by method of Stiles, Peterson, and Fred.

3. Total sugar, 60-minute converted mashes. Centrifuge or filter sample of excess fermenter mash. Hydrolyze with hydrochloric acid as above and run sugar.

d. Balling. Determine on the undiluted supernatant with an 8° to 16° Balling hydrometer.

(7) FINAL DATA. *Preparation of Sample.* Mix the fermented mash thoroughly, weigh the fermenter, and calculate the weight of mash. Weigh exactly one half of the mash into a 1-liter distillation flask and add 50 per cent of the water in the trap to this sample, which is used for whole mash alcohol analyses for proof gallon per bushel yield calculations as follows (proof gallon per bushel is a term used to denote gallons of 100° proof alcohol yield from 1 bushel of grain. In distilling practice corn, rye, wheat, and malt are measured in 56-pound bushels. The bushel is measured on an air-dry basis unless otherwise stated.)

a. Distill into a 100-cc. volumetric flask with 5 cc. of water in the bottom. The condenser should be provided with a tip so that all vapors pass through the water in the receiver. (Receiver should be immersed in an ice bath.) Distill up to neck of volumetric receiving flask.

b. Warm distillate to room temperature, make up to volume, and determine alcohol with the immersion refractometer (usually used at 17.5° C.).

Centrifuge the remainder of the mash at 2000 r. p. m. for 10 minutes and analyze the supernatant as follows:

a. pH, as initial.

b. Titratable acidity, as initial.

c. Balling. Final Ballings should be determined with a Balling hydrometer, range −2.0° to +2.0°, of such size that it may be used in a 50-cc. graduate for convenience.

d. Final sugar. Place a 5-cc. sample in a 25 × 200 mm. Pyrex test tube, add 5 cc. of 1.38 *N* hydrochloric acid, and hydrolyze the sample in a boiling water bath for 2.5 hours. Determine the sugar content, calculated as glucose, by the method of Stiles, Peterson, and Fred (*2*).

e. Alcohol for efficiency calculation by Method B. Pipet 50 cc. (volumetric pipet) into distillation flask, add enough 0.5 *N* sodium hydroxide calculated from titratable acidity to neutralize beer, and add 1 cc. of mineral oil to prevent foaming. Distill over 50 per cent of total volume into 50-cc. volumetric flask, make to volume, and determine alcohol as in proof gallon per bushel analysis.

Calculations

1. CALCULATION OF PROOF GALLON PER BUSHEL. Proof gallons per bushel = (per cent by volume of alcohol as read in 100 cc. of distillate) − (initial sugar concentration of yeast mash as grams of maltose per 100 cc. × 0.0208) ×

$$\frac{100 \text{ (cc. of distillate)}}{175 \text{ (cc. of mash distilled)}} \times \text{concentration of mash (gallons of mash per bushel)} \times \frac{2}{100}$$

For calculation of proof gallons per bushel from fermentations made by the process described above, in which the mash concentration is 38 gallons per bushel, the factor is as follows: Proof gallons per bushel = (per cent by volume of alcohol as read) − (initial sugar concentration of yeast mash as grams of maltose per 100 cc. × 0.0208) × 0.4343.

The proof gallons per bushel yield should be expressed on a dry basis for comparison. This is determined by dividing the above figure by the per cent of dry grain mashed.

2. DERIVATION OF CORRECTED FACTOR FOR YEAST MASH IN PROOF GALLONS PER BUSHEL ALCOHOL ANALYSIS.

y = sugar concentration of yeast mash (grams per 100 cc.).

$0.511y$ = grams of absolute alcohol from 100 cc. of yeast mash.

$0.511y \times 0.035$ = grams of absolute alcohol from 3.5 cc. of yeast mash.

$(0.511y \times 0.035)\ 0.92$ = grams of absolute alcohol from 3.5 cc. of yeast mash, assuming 92 per cent efficiency.

$\frac{0.511y \times 0.035 \times 0.92}{0.791}$ = per cent of alcohol in 100 cc. of distillate due to yeast mash.

∴ Correction factor = $0.0208y$.

3. CALCULATIONS OF EFFICIENCY. *Method A* (based on starch analysis). The composite starch content of the grain bill is calculated in pounds per dry bushel of grain by weighted averages.

Table II. Initial Data

Cook No.	pH	Balling	Titration of Acid	Initial Sugar	Maltose	Conversion	Moisture of Corn
			Cc. 1 N NaOH / 10 cc.	*G./100 cc.*	*G./100 cc.*	%	%
1	4.83	11.6	1.82	12.08	8.44	70.6	13.41
2	4.86	11.9	1.88	12.03	8.75	73.5	13.41

Table III. Efficiency of Fermenter

Cook No.	Fermentation No.	pH	Titration of Acid	Balling	Final Sugar	Wet	Dry	Alcohol	Plant Efficiency	Fermentation Efficiency
			Cc. 1 N NaOH/ 10 cc.		*G./100 cc.*	*Proof gal./bu.*		*% by vol.*	%	%
1	4	4.18	4.80	−0.5	0.60	5.48	6.32	7.19	92.2	97.0
	12	4.16	4.81	−0.5	0.60	5.47	6.31	7.16	91.7	96.5
	131	4.16	4.70	−0.6	0.59	5.46	6.30	7.17	91.8	96.7
2	10	4.34	3.95	−0.5	0.68	5.54	6.38	7.23	92.9	98.0
	27	4.32	3.82	−0.4	0.68	5.54	6.38	7.24	93.1	98.2
	111	4.36	4.22	−0.5	0.68	5.51	6.35	7.22	92.8	97.9

Pounds of starch × 0.1725 = theoretical proof gallons per bushel (0.1725 is the factor for conversion of pounds of starch to proof gallons of alcohol).

$$\frac{\text{Actual yield (dry basis)}}{\text{theoretical (dry basis)}} \times 100 = \text{per cent efficiency}$$

Method B (based on sugar analysis).

a. Fermentation efficiency (based on sugar fermented)

$$\frac{\text{Actual grams of absolute alcohol obtained}}{(\text{initial total sugar} - \text{final sugar})\ 0.511} \times 100 = \text{per cent fermentation efficiency}$$

b. Plant efficiency (based on total sugar present)

$$\frac{\text{Actual grams of absolute alcohol obtained}}{\text{initial total sugar} \times 0.511} \times 100 = \text{per cent plant efficiency}$$

c. Grams of absolute alcohol per 100 cc. = per cent of alcohol × 0.791

Sample Calculations. The calculation of the results of experiments presented in Tables II and III is shown in detail in the following examples:

Example I. Method B was used for calculating efficiencies.

1. Calculation of proof gallons per bushel yield from fermenter No. 4 (Table II).

Initial sugar on yeast mash = 15.60 grams per 100 cc.

Alcohol as read in 100 cc. of distillate distilled from 0.5 volume of mash in fermenter = 12.93 per cent.

12.93 − (15.60 × 0.0208) × 0.4343 = 5.48 proof gallons per bushel (wet basis)

$$\therefore \frac{5.48}{86.59} \times 100 = 6.32 \text{ proof gallons per bushel (dry basis)}$$

2. Method B. Calculation of efficiency of fermenter No. 4 (Table III).

a. Fermentation efficiency. Per cent of alcohol by volume = 7.19.

7.19 × 0.791 = 5.69 grams of absolute alcohol per 100 cc.

$$\frac{5.69}{(12.08 \times 0.60)\ 0.511} \times 100 = 97.0 \text{ per cent fermentation efficiency}$$

b. Plant efficiency.

$$\frac{5.69}{12.08 \times 0.511} \times 100 = 92.2 \text{ per cent plant efficiency}$$

Example II. Calculation of Efficiency by Method A. In this case only starch, moisture, and proof gallons per bushel alcohol (whole mash alcohol) data are obtained.

Grain bill, 92.0 per cent corn, 8.0 per cent barley malt

Starch content of corn = 61 per cent (wet basis). Moisture of corn = 11.5 per cent.

Starch content of malt = 51.5 per cent (wet basis). Moisture of malt = 7.0 per cent.

Composite moisture (by weighted average) = 11.18 per cent.

Composite starch content of grain bill in pounds per dry bushel

$$= \frac{\left(92 \times \frac{61}{0.885}\right) + \left(8 \times \frac{51.5}{0.93}\right)}{100} = 67.91 \text{ (dry basis)}$$

Actual alcohol yield, 5.33 proof gallons per bushel (wet basis) and 6.00 proof gallons per bushel (dry basis). 67.91 × 56 × 0.1725 = 6.56 theoretical proof gallons per bushel.

$$\text{Per cent efficiency} = \frac{6.00}{6.56} \times 100 = 91.4$$

Discussion

Either the complete method or one of the three alternates listed below may be used, dependent on the factor under investigation.

Starch analysis of grain with determination of proof gallons per bushel alcohols, no micro sugars or alcohols. Efficiency is calculated by Method A.

No starch analysis, proof gallons per bushel alcohols determined as well as efficiency alcohols and micro sugars. Efficiency is calculated by Method B.

No proof gallons per bushel alcohols, no starch analysis, and results evaluated only by efficiency as calculated by Method B.

This represents the standard or control procedure. It is used when evaluating grains, yeast strains, or yeast propagation and is run as a comparison if some condition within the procedure itself is under investigation.

Interpretation of Results

As with all new laboratory procedure, the analyst should run a sufficient number of control tests to determine his ability to secure good fermentations and reproducible results before attempting to introduce any variables. The criteria of a good fermentation are as follows:

Corn Mash. *Initial Sugar*, about 12 grams per 100 cc. Low sugar indicates poor cooking or poor grain.

Per Cent Conversion, about 70. Low per cent conversion may indicate poor cooking or poor grade of malt.

Final pH, above 4.0. A low final pH is the result of bacterial contamination.

Titratable Acidity, 3.5 to 4.5. High acidity, like low pH, indicates bacterial contamination.

Final Balling, 0.3 to 0.7. A high final balling indicates incomplete fermentation.

Proof Gallons per Bushel Yield, 5.2 or above.

Plant Efficiency, 92 per cent to 94 per cent.

Wheat Mash. The above limits also hold good for wheat mashes, except that final Ballings are often somewhat higher and the final yield is usually lower (5.0 to 5.1 proof gallons per bushel).

Rye Mash. Because of the viscosity of rye mash, the final Balling is unreliable as an index to the completeness of fermentation. The yield from a rye fermentation should be 4.8 proof gallons per bushel or above.

Acknowledgments

The authors wish to thank F. H. Gallagher and W. Payne for their contributions toward the development of this procedure, G. A. Ratti for the temperature control boxes employed, and A. F. Novak for the illustrations.

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Analysis of Butane-Isobutane Mixture

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MIXTURES of *n*-butane and isobutane, such as might result from isomerization processes or from a preliminary fractional distillation of natural or casinghead gas, are commonly analyzed by low-temperature fractional distillation, as with a Podbielniak still. For accurate results this requires a trained technique, at least 3 hours' time, and at least 10 grams of sample. For control purposes the time factor especially is a detriment.

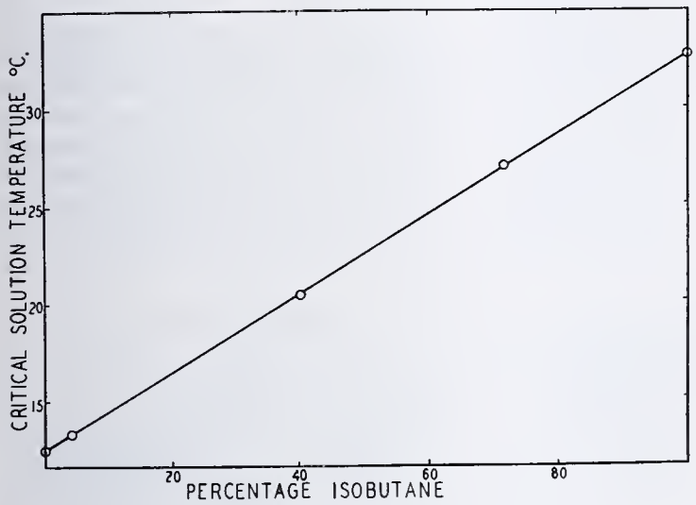

FIGURE 1

Alternative methods proposed are the percentage of a gaseous mixture condensed at dry ice temperatures (*5*), the shape of the pressure-volume isothermal curve (*7*), the index of refraction at −25° C. (*4*), and Raman spectra (*3*). The first two seem crude and inaccurate, the third requires special technique, and the last requires expensive apparatus. Another method tried is the temperature of disappearance of the interface between the liquefied butanes and water (19° C. for *n*-butane, 3° C. for isobutane, *1*), but it was found difficult to observe this temperature with sufficient precision.

The method here proposed (*2*) is the observation of the critical solution temperature of the butane mixture with pure *o*-nitrotoluene.

TABLE I. COMPOSITION OF BUTANES

Critical Solution Temperature	Isobutane Present	Isobutane Found
° C.	%	%
12.5	0	0
13.3	4	4.0
20.5	39.8	39.4
27.0	71.4	71.4
32.8	100	100

Apparatus and Procedure

The apparatus consists of glass tubes or ampoules, 10 to 20 cm. long and 5 to 8 mm. in diameter, sealed at one end and drawn down to a narrow neck at the other, about 1 to 2 mm. in diameter. These are filled about 30 per cent full with *o*-nitrotoluene through a capillary funnel, and immersed in a cold bath of dry ice and acetone (which freezes the *o*-nitrotoluene). A sample of butanes to be analyzed is condensed into a tube through a capillary funnel, filling the tube to about 1.7 times the depth of the solid *o*-nitrotoluene—i. e., the tube is about 75 per cent filled.

The tube is then sealed at the neck with the fine flame of a blast lamp while the tube is still immersed in the cold bath. The tube is attached by rubber bands to a thermometer reading preferably in fractions of a degree, and immersed in a water bath such as a large glass test tube. By frequent tipping of the tube with gradually rising temperature or twirling with falling temperature, the point of disappearance or reappearance of cloudiness can be observed within 0.1° or 0.2° C., corresponding to less than 1 per cent error in percentage of isobutane. The test requires 10 to 15 minutes.

The composition of the butanes is a linear function of the critical solution temperature, as illustrated in Table I and Figure 1.

Other solvents could be used in place of *o*-nitrotoluene, as shown in Table II.

The high freezing point of acetophenone, 19.7° C. makes it less convenient, since it sometimes crystallizes out during a determination; and the higher critical solution temperature with isobutane in the case of the other solvents means a higher pressure and greater risk of breaking the tubes, as well as less precise temperature reading. Moreover, cresol and eugenol are less readily available in pure form.

TABLE II. CRITICAL SOLUTION TEMPERATURE

Solvent	*n*-Butane	Isobutane
	° C.	° C.
Acetophenone	10.6	24.5
o-Chloroaniline	29.8	50.5
Cresol (technical)	14.2	45.5
Eugenol	23.0	43.0
Nitrobenzene	40.0	61.0
Aniline (*6*)	84.1	109.0

TABLE III. CRITICAL SOLUTION TEMPERATURES WITH AROMATIC SOLVENTS

Paraffin	*o*-Nitrotoluene	Nitrobenzene	Aniline
	° C.	° C.	° C.
Propane	65	None	None
Isobutane	32.8	61	109
n-Butane	12.5	40	84.1
Neopentane	30[a]	54[a]	102[a]
Isopentane	9	32	78.4
n-Pentane	2	24	71.5
Neohexane	11[a]	33[a]	81
n-Hexane	−1	21	69.1

[a] Estimated.

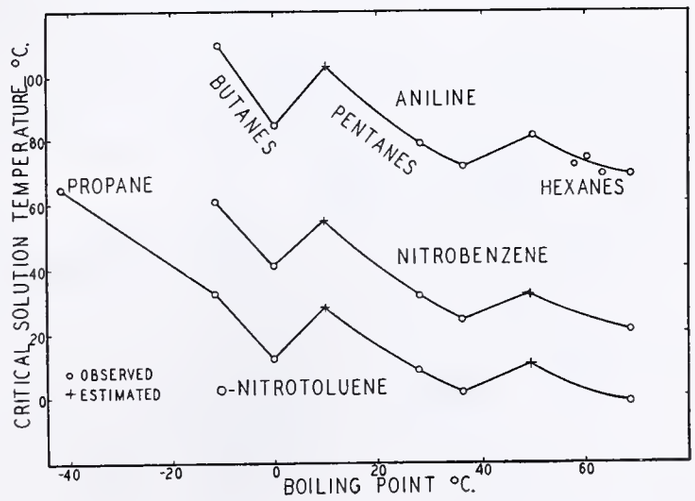

FIGURE 2

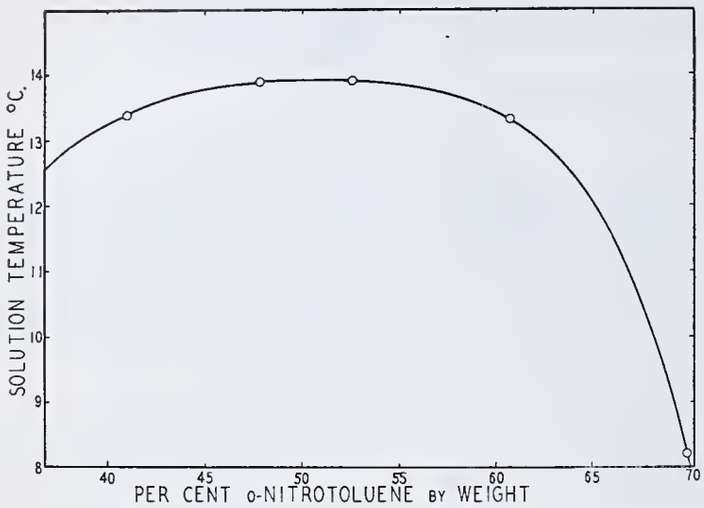

FIGURE 3

Effects of Contaminants

The method is intended for analysis of a wholly butane mixture. Olefins must be absent, since a small percentage would lower the critical solution temperature considerably. This condition is met readily as isomerization catalysts destroy any olefin gas present. Presence of water in the sample is objectionable—it freezes and plugs up the capillary funnel, but it does not affect the critical solution temperature, probably because it is so slightly miscible with either the nitrotoluene or the butane. It can be removed with a calcium chloride drying tube in the gas line before condensation.

The presence of other paraffins affects the determination to some extent, as illustrated in Table III and Figure 2.

Thus the presence of 1 per cent propane in the "butane mixture" would cause an error of about 2.6 per cent isobutane too high. One per cent of isopentane would make the percentage of isobutane only 0.2 per cent too low, and a similar amount of *n*-pentane about 0.5 per cent too low. If the amounts of these paraffin impurities are sufficient to interfere with the determination, it is probably necessary to run a distillation analysis. Table III and Figure 2 include the other two solvents to show the remarkable parallelism for different aromatic solvents. Propane is omitted in those cases because its critical temperature is reached without mixing with aniline (*1*) or nitrobenzene. The aniline critical solution temperature of neopentane was estimated in a previous paper (*1*), and the other estimates were made by paralleling those with aniline.

The effect of ratio of solvent to hydrocarbon is shown in Figure 3, observed with a sample of butane containing 7 per cent of isobutane. If the percentage of *o*-nitrotoluene is between 45 and 55 per cent (30 to 40 per cent by volume) the solution temperature is not over 0.1° below the critical solution temperature. A mixture outside these limits in composition would be noticed readily, since the final position of the interface would be far from the middle of the system.

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Vacuum Sublimation

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APPARATUS for sublimation on a macro scale is seldom encountered in chemical laboratories. Almost all sublimation apparatus described in the literature is designed for micro work (*2*, *3*). Sublimation in vacuo is an excellent method for purifying compounds and is worthy of greater consideration. The advantage of this method over crystallization is that the loss of material is negligible, the sublimed compound is dry and free from solvents, and the actual labor involved is small. In many cases, one compound can be separated from a complex mixture by sublimation much more quickly than by the conventional method of extraction. Naturally, the method has its limitations—e. g., compounds having the same rate of sublimation cannot be separated, and some substances, when heated to their sublimation temperatures, undergo decomposition.

When sublimation is applied to routine analysis and research in the laboratory, under controlled conditions, a permanent setup is desirable. As a heat source, a resistance wire wound around the sublimation tube (*4*) or a small copper heating block with a hole for the glass tube (*1*) has been recommended, but such devices do not maintain constant temperatures without a thermostat. Most important in sublimation experiments is means for heating the tubes to a definite and constant temperature. The author does not claim originality, but describes a unit consisting of a heating block and sublimation tubes which have given good service in this laboratory.

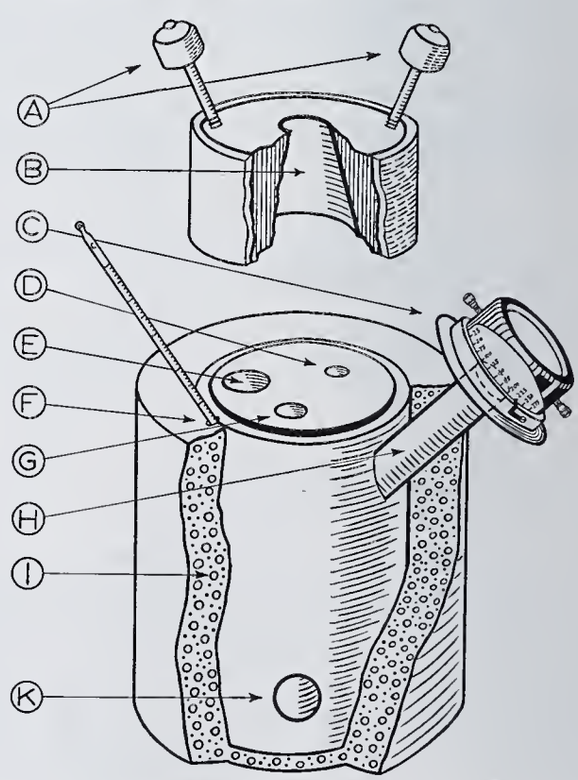

FIGURE 1. HEATING BLOCK

Originally, a heating block to accommodate but one size of tube was used, but later an improved block was constructed for a range from room temperature to 275° C. To enhance the utility of this block, three holes were drilled for three different sizes of tubes, and an auxiliary top was provided, so that 25-ml. and 50-ml. Erlenmeyer flasks could be heated to specified temperatures. It can also be used as a constant heat source for laboratory apparatus.

Two tables show the application of vacuum sublimation. In Table I, the rate of sublimation of various compounds under definite conditions is given. Among the samples are a dye, several "sulfa" compounds, an amino acid, and vitamin C. The quantitative determination of active ingredients in tablets has been found to be feasible. The analytical data are compiled in Table II.

Table I. Rate of Sublimation

Compound (0.200 Gram)	Temperature ° C.	Pressure Microns	Time Hours	Sublimate Weight Gram	Sublimate Melting point[a] ° C.
Salol	35	9	6	0.192	41.7– 42.2
Azobenzene	40	9	2	0.155	67.5– 68.5
8-Hydroxyquinoline	40	8	1	0.154	73.5– 74.2
2-Methyl-1,4-naphthoquinone	60	7	2	0.184	105.4–106.3
	80	8	0.5	0.189	
2-Hydroxy-3-methyl-1,4-naphthoquinone	80	11	3	0.191	172.6–172.9
Nicotinamide	80	6	5	0.183	128.2–129.9
Pyramidon	90	15	1.5	0.200	106.0–106.5
Acetylsalicylic acid	100	10	1	0.198	130.4–131.5[b]
Phenacetin	100	13	1.5	0.167	134.1–134.8
Caffeine	120	8	1	0.167	236.2–236.8
1-Hydroxyanthraquinone	130	9	0.33	0.165	194.4–195.2
2-Hydroxyanthraquinone	130	7	24	0.063	310.5–311.1
1,8-Dihydroxyanthraquinone or Istin	130	10	1.5	0.171	193.6–194.1
Saccharin	130	10	2	0.195	225.2–227.8
Phenobarbital or 5-ethyl-5-phenyl-barbituric acid	140	9	1.5	0.199	175.7–176.3
Pentaerythrite	150	10	3	0.193	256.4–260.6
Fluoran	150	12	1.5	0.181	183.8–184.3
Vitamin C or ascorbic acid[c]	150	8	24	0.052	186 –187[d]
l-Leucine	150	8	10	0.139	287.0–288.2
D. C. Yellow No. 11 or 2-(2-quinolyl)-1,3-indandione	150	5	20	0.189	242.3–242.8
Sulfanilamide[e]	120	4	24	0.170	164.6–165.2
	150	5	2	0.199	
Sulfaguanidine[f]	150	8	24	0.001	183.6–185.0
Sulfapyridine	150	6	10	0.108	190.3–191.1
Sulfathiazole[g]	150	7	24	0.015	192.1–198.7
Sulfadiazine[h]	150	6	24	0.038	252.2–252.8
Phenolphthalein	180	7	24	0.072	260.6–261.5
Quercetin	200	7	16	0.042	317.4–317.9

[a] All melting points are corrected and were determined in a Hershberg precision melting point apparatus.
[b] Same m. p. as that obtained in same bath on a sample of pure acetylsalicylic acid.
[c] Vitamin C courtesy of Chas. Pfizer & Co., Inc.
[d] Same m. p. as that obtained in same bath on a sample of pure ascorbic acid. Nonsublimed part had become brownish and melted 1° lower.
[e] Sulfa compounds and tablets courtesy of Maurice E. Avery, Lederle Laboratories, Pearl River, N. Y.
[f] Sulfaguanidine charged melted at 187.6–188.7°, nonsublimed part at 189.6–190.5°.
[g] Sulfathiazole charged melted at 200.0–200.7°, nonsublimed part at 199.9–201.0°.
[h] Sulfadiazine charged melted at 254.5–254.8°, nonsublimed part at 253.9–254.5°.

The Apparatus

The heating block (Figure 1) was made from a cast cylinder of 85 per cent copper and 15 per cent tin, 100 mm. in diameter and 150 mm. high. Three holes drilled on top for three different sizes of test tubes are of the following dimensions: $E = 23 \times 90$ mm., $G = 14 \times 80$ mm., and $D = 11 \times 62$ mm. Thermometer well F, 8×82 mm., is inclined towards the center. A hole with an extension tube, H, 19 mm. in inside diameter, is likewise inclined towards the back, leaving the front free for working. H is threaded on the main block and takes the thermostat, C. Hole K, 20×70 mm., is for the heating unit.

To enable one to heat 25-ml. and 50-ml. Erlenmeyers, an auxiliary top was added, 100 mm. in diameter and 55 mm. high. This top is recessed to fit over the main block and by means of two wooden knobs, A, can be removed easily. Conical hole B has a top diameter of 25 mm. and a bottom diameter of 52 mm.

The block is lagged on the bottom and on the sides by a 30-mm. layer of magnesia, I. The side of the auxiliary block is protected by a layer of asbestos paper.

The electrical parts consist of a G-E cartridge-type heater, 200-watt, 115-volt, No. 151-H, 1.88 cm. (0.75 inch) in diameter and 5.9 cm. (2.375 inches) long, fitting into K; a Quick-Set bimetal thermoregulator, C, range 25° to 275° C. (No. 4-239, American Instrument Co., Silver Springs, Md.) fits into tube H, which is 205 mm. deep; a relay, 115-volt (No. ABYT 8, Struthers, Dunn, Inc., Philadelphia, Penna.), is placed in a wooden box and provided with the necessary electrical connections to the heating unit and the thermoregulator.

Table III shows the performance of the heating block.

The sublimation tubes are shown in Figure 2. Tube B is air-cooled; A and C have an inner water-cooled condenser on which the sublimate collects. The tubes fit into hole E (Figure 1).

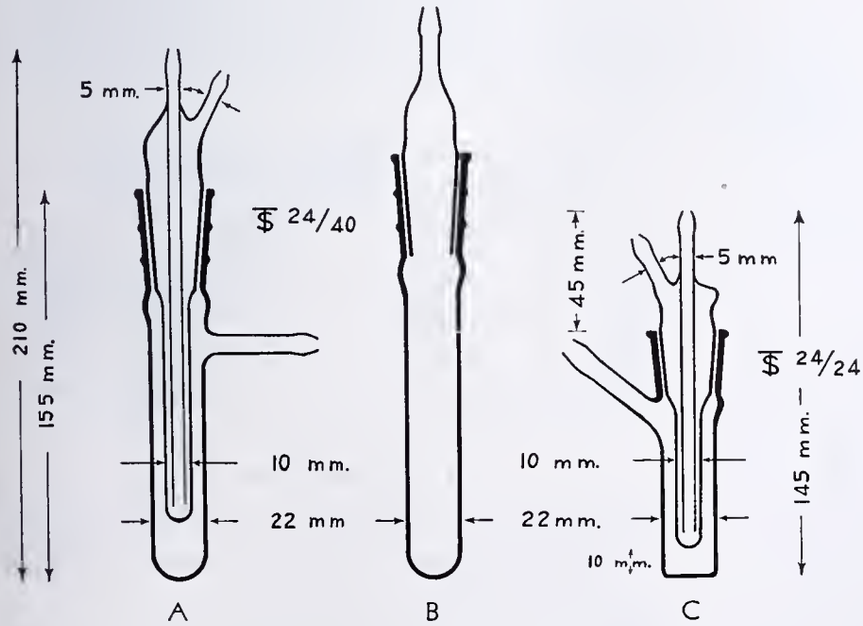

Figure 2. Sublimation Tubes

Method

The air-cooled sublimation tube, B, is used mainly in cases where two sublimates are expected.

For example, when a mixture of benzoic acid and *o*-benzoyl-benzoic acid is sublimed, two crystalline sublimates are obtained, each forming a separate band on the cooler or the part of the tube which extends out of the heating block. Each ring is then scraped out separately. In order to determine whether the sublimation is at an end, the entire tube is pulled out about 1 cm. without interrupting the sublimation. If incomplete, a new band of sublimate will form farther inside the old ring.

Sublimation tube A is preferred when only one sublimate is expected. This tube will accommodate up to 2 grams. It is also possible to conduct fractional sublimations by using either this tube or tube C.

For example, a mixture of 1- and 2-hydroxyanthraquinone can be separated more easily and with practically no loss of material by first keeping the tube at 100° C. and 10 microns.

Table II. Analytical Data

Composition of Sample[a]	Weight of Sample	Sublimation			Sublimate				
		Temperature	Time	Pressure	Weight average of (15 determinations)	Melting Point[a]	Percentage		
							Min.	Max.	Average and mean deviation
	Gram	° *C.*	*Hours*	*Microns*	*Grams*	° *C.*			
50.00% salol, m. p. 41.7–42.2°, and 50.00% phenacetin, m. p. 134.0–134.5°	0.0500	35	2	11	0.0248	41.1– 41.8	48.0	50.8	49.60 ± 0.56
		120	0.25	13	0.0252	132.2–134.2	50.0	52.0	50.41 ± 0.28
2.22% 2-methyl-1,4-naphthoquinone, m. p. 104.6–106.1° (also lactose, calcium gluconate, starch, sucrose, and small quantities of talcum and magnesium stearate)	0.5000	80	0.5	14	0.0107	104.8–105.8	2.04	2.26	2.14 ± 0.05
84.29% acetylsalicylic acid and starch	0.0500	110	0.33	13	0.0422	132 –133[b]	83.8	85.2	84.40 ± 0.34
33.31% nicotinamide, m. p. 128.8–129.4° (also lactose, starch, sucrose, and small quantities of talcum and magnesium stearate)	0.0500	110	0.33	11	0.0169	128.5–129.0	33.0	34.2	33.77 ± 0.30
75.00% phenacetin, m. p. 134.2–134.8° (also sucrose, starch, and small quantities of talcum and magnesium stearate)	0.0500	120	0.5	10	0.0376	133.9–134.6	74.6	76.0	75.25 ± 0.40
46.29% phenobarbituric acid, m. p. 175.4–176.4°, and 1.46% stearic acid and starch	0.1000	150	0.67	11	0.0482	173.6–175.1	46.8	49.5	48.17 ± 0.57
81.20% sulfanilamide, m. p. 164.4–164.8° (also starch, sucrose, and small quantities of talcum and magnesium stearate)	0.1000	155	3	10	0.0815	164.3–164.8	80.7	82.2	81.56 ± 0.31

[a] All melting points are corrected.
[b] Same m. p. as that obtained in same bath on a sample of pure acetylsalicylic acid.

The sublimate will be the more volatile 1-hydroxyanthraquinone. The experiment is now interrupted and the first sublimate is collected. The temperature of the heating block is then raised to 180°, or a second heating block previously heated to this temperature is used, and the sublimation is continued. The sublimate which now forms is the 2-isomer. The separation of the two isomers by this method is much sharper than by fractional crystallization.

Another example of fractional sublimation, the mixture of salol and phenacetin, will be found in Table II. Salol was first sublimed from the mixture at 35° C. and then the phenacetin was sublimed at 120°.

The second water-cooled tube, *C*, is used for smaller samples and mainly for quantitative determinations.

It has a flat bottom, so that the substance is exposed in a thin layer. The distance between the bottom and the condenser tip is but 10 mm.; therefore, the vapors have but a small distance to travel. The inside condenser weighs 23 grams and is small enough to be hung up on a balance arm by means of a wire attached to the constriction. The sublimate will collect on the tip of the condenser and will adhere firmly, as was observed by Kempf (*3*). There need be no fear that the sublimate will fall off when handling the condenser. When the clean ground joints are lubricated with Cenco Vacu-Seal, they will never stick.

To illustrate the variation in the rates of sublimation, data on twenty-seven organic compounds are shown in Table I.

The figures were obtained as follows: 0.200 gram of the compound (purest grade available) was placed on the bottom of tube *A* and then connected with the vacuum pump. As soon as the necessary low pressure was reached, the tube was placed in hole *E* of the heating block. The pressure was measured every hour or more on a McLeod gage. The average reading is shown in Tables I and II. The melting points of each compound before sublimation, of the sublimate, and of the part not sublimed, were determined. There was no decomposition observed except in the cases mentioned. Substances which did not sublime at 150° C. and 10 microns were: starch, sucrose, lactose, talcum, magnesium stearate, 1-cystine, and *d*-glutamic acid.

Sublimation is used in this laboratory for the quantitative determination of active ingredients in pharmaceutical tablets. The method may not be so accurate as some others, but it is time-saving in many instances, and the compound is obtained in dry and pure form suitable for identity tests such as mixed melting point determinations, etc. The results are slightly high, but no explanation for this can be advanced now. The estimation of 2-methyl-1,4-naphthoquinone, acetylsalicylic acid, phenacetin, phenobarbital, nicotinamide, salol, and sulfanilamide in tablets has been found to be practical. The same method could not be applied to sulfapyridine, sulfaguanidine, sulfathiazole, and sulfadiazine tablets, because these four sulfa compounds do not sublime to any extent below 160° C. at 10 microns pressure; and above that temperature, some of the other tablet constituents decompose slowly. The data in Table II were obtained as follows:

A mixture containing the same percentage of ingredients as in commercial tablets was prepared on an analytical balance. A weighed sample of from 0.05 to 0.50 gram was placed on the flat bottom of tube *C* and was spread evenly by tapping. The temperature of the cooling water varied from 9° to 17° C. The time required for complete sublimation of the compound in question from the mixture had previously been determined, and in the actual quantitative determination, the time was lengthened by one third to assure complete sublimation. At the end of the procedure, the condenser was removed, most of the water was blown out of the condenser, the two inlet tubes were stoppered, and the sealing wax was wiped from the ground surface. The condenser with the adhering sublimate was weighed, the sublimate then scraped off, the tube tip wiped clean with a solvent, and the condenser minus the sublimate weighed again.

The phenobarbital tablets contain 1.46 per cent of stearic acid; therefore the sublimate will be a mixture of 97 per cent phenobarbital and 3 per cent of stearic acid. In this mixture the phenobarbital may be estimated by one of the standard methods. Other common tablet ingredients like sucrose, lactose, starch, talcum, and magnesium stearate do not sublime under the conditions indicated in Table II. Equally good results were obtained when finished tablets, first reduced to a powder, were analyzed by this sublimation method.

Table III. Performance of Heating Block

Temperature	Temperature Reached within:	Fluctuations of Temperature	Power Consumption
° *C.*	*Min.*	° *C.*	*Watts*
100	28	±0.3	23
150	49	±0.3	46
200	72	±0.4	69
275	132	±0.5	114

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Estimation of Water in Alcohol with Aid of Dicyclohexyl

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Water content in otherwise pure ethyl alcohol of nearly absolute grade may be determined by measurement of critical solution temperature in the system alcohol-water-dicyclohexyl. The "dicyclohexyl point", easily obtained in a few minutes without necessity of standard solutions or special apparatus, is referred to a graph for percentage value.

THE critical solution temperature of a kerosene-alcohol mixture serves as a remarkably precise index of water content, as reported by Crismer (*3*), Andrews (*2*), and the Bureau of Standards (*5*). Each lot of kerosene, however, must be calibrated by a laborious process. The method is, therefore, of little use to the majority of laboratory workers requiring only occasional determinations of water in alcohol. A single substance, similar in physical properties to kerosene but which could be calibrated once for all, would be a desirable substitute. For this purpose a paraffin hydrocarbon with molecular weight of about 170 would be ideal—for example, a dodecane. Unfortunately, pure open-chain paraffins of such high molecular weight are available only as costly academic curiosities.

A new solution of this problem has become possible through the commercial appearance of dicyclohexyl (bicyclohexyl, dodecahydrodiphenyl). The system ethanol-dicyclohexyl has the convenient critical solution temperature of 23.4° C., with an elevation of 18° for the first 1 per cent of water added to the alcohol so tested. Provided any given alcohol preparation and the dicyclohexyl are mixed in or near the ratio of critical composition during calibration, no great precision in measurement of volumes is required in subsequent determinations, as shown in Figure 1. Since the main aim of this work is to test alcohol that is nearly absolute, the simple volume ratio of 1 to 2 was chosen for the final graph of Figure 2.

Sources of Error

The shift of peak of the miscibility curve toward the dicyclohexyl axis with increase of water content calls for increasing care in measurement of volume, and the probable error grows. Since the precise determination of water in alcohol over the range 96 to 99 per cent (by weight) seems to be an unimportant problem, no attempt is made here to complicate matters by reporting data at other volume ratios than the uniform 1 to 2 value adopted. Determinations below 99 per cent are thus only approximate.

Unfortunately, mixtures of dicyclohexyl and alcohol, either of the critical composition or nearly that ratio, markedly display the phenomenon of critical opalescence (*8*) at temperatures just above the maximum temperature of genuine turbidity. This cuts down slightly the extreme precision characteristic of the Crismer technique using kerosene. With dicyclohexyl and a good thermometer it is nevertheless easy to distinguish alcohol preparations as close to each other, for example, as 99.90 and 99.91 per cent; by the Crismer method, 99.900 and 99.903 per cent.

More important is the problem of purity of the dicyclohexyl. Fortunately, the present industrial product is a synthetic individual of high grade, derived by hydrogenation of diphenyl. Several lots of the hydrocarbon, both "technical" and purified, were tested against a standardized alcohol of 99.9+ per cent grade. No significant difference in critical solution temperature was found in this series; certainly

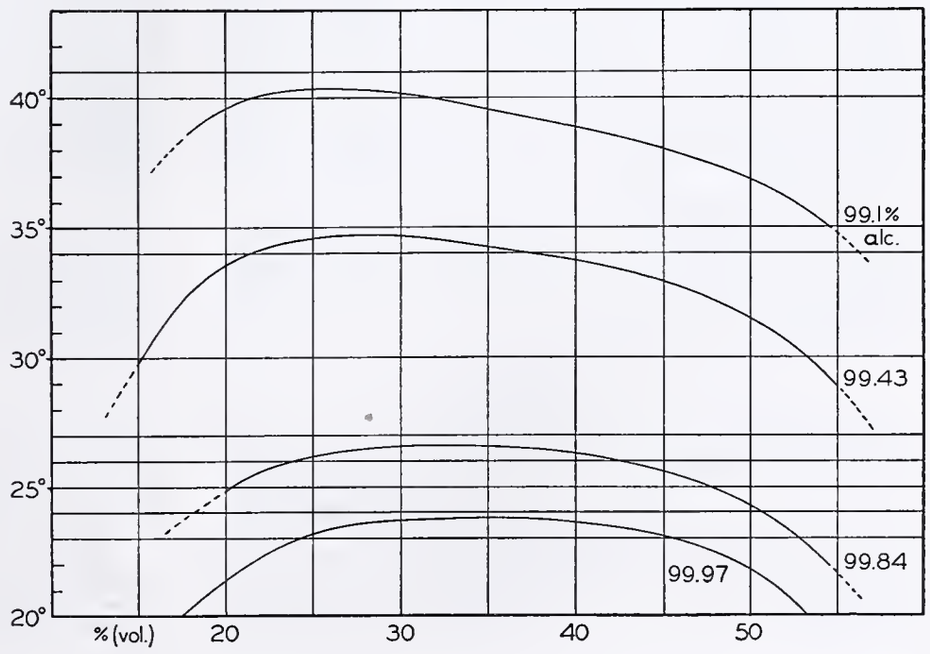

FIGURE 1. MAXIMUM TEMPERATURES OF TURBIDITY

Solutions of aqueous ethyl alcohol preparations in dicyclohexyl. Abscissa, volume per cent alcohol

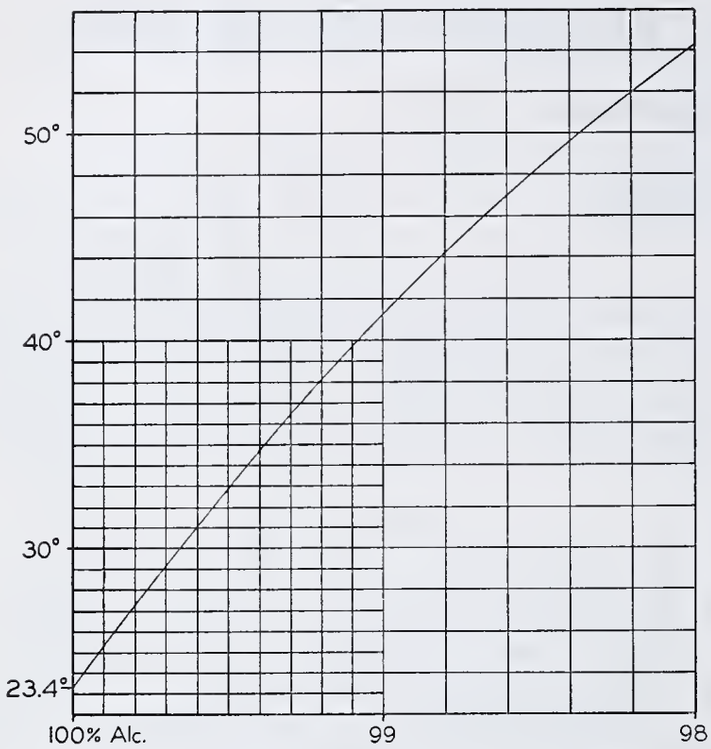

FIGURE 2. PERCENTAGES OF ETHYL ALCOHOL AND CORRESPONDING DICYCLOHEXYL POINTS
1 to 2 alcohol-dicyclohexyl ratio

none equivalent to the difference between 99.90 and 99.91 per cent alcohol.

Water content in the dicyclohexyl is not a serious problem. Simple filtration of the clear liquid product through dry filter paper removes all stray suspended globules of water, and perhaps even some of the extremely small content of dissolved water. The data used in preparing Figure 2 refer to clear samples of the hydrocarbon which have been exposed freely to ordinary atmospheric conditions. When such material was thoroughly dried, no significant change in melting point could be detected with a Beckman thermometer.

A series of purified alcohol preparations (98 to 99.99 per cent), the densities of which at 25.00° were determined with a graduated pycnometer (*6*), was first used to standardize a special paraffin oil by the Crismer method. The oil consisted of highly refined kerosene to which had been added enough colorless paraffin oil to raise the critical solution temperature values out of ranges requiring an ice bath, and to a region close to the critical solution temperature values for dicyclohexyl. Once this paraffin oil standard was established, cross comparisons of an extended series of alcohol samples, prepared in random concentrations, were easily made, each with the two hydrocarbon preparations, oil and dicyclohexyl.

The miscibility-temperature determinations were made in a short 20-mm. test tube with siphon drain from the bottom. This device was fitted with thermometer, micromechanical stirrer, burets, and inlet for desiccated air. The alcohol supply was admitted from a distillation receiver without intermediate exposure to the outer atmosphere.

Materials Used

PARAFFIN OIL. "Elaine" kerosene (Standard Oil Co. of Calif.), 7 volumes. Standard White Oil No. 7 (Standard Oil Co. of Calif.), 1 volume. Such a mixture yields a critical solution temperature, with 100 per cent alcohol, in the vicinity of 23° C.

ETHYL ALCOHOL. Commercial 95 per cent fermented spirit was given three reflux treatments over quicklime, each followed by distillation with substantial rejections of foreruns and tailings to eliminate acetaldehyde and higher alcohols. In one case the rapid and extremely convenient Adickes (*1*) method, employing ethyl formate, was used in the final operation with concordant results. Particular attention was paid to the most highly dehydrated alcohol which was attainable. Six lots, prepared on different days by the two methods, had critical solution temperature values (with the paraffin oil) varying from 23.27° to 23.37°, and densities at 25.00° C. from 0.78506 to 0.78508, there being no consistency nor correlation within those ranges; average 23.31° and 0.78507+. By short extrapolation, 23.2° was taken as the critical solution temperature for 100 per cent alcohol, for which the extremely reliable density value of 0.78506 is known (*5*).

DICYCLOHEXYL. Three lots of industrial origin were investigated:

	Melting Point ° C.
1. Eastman Kodak Co., P4641, merchandise of 1941	3.5
2. Dow Chemical Co., technical product of 1943	3.4
3. Special preparation made by fractionation of 2 through a 30-plate column, followed by recrystallization	3.63

The melting-point value for No. 3 refers to the constant equilibrium temperature of a mush of the hydrocarbon alternately freezing and melting slowly in a bath varied from 3° to 4°, there being from $^1/_3$ to $^2/_3$ of solid present. A National Bureau of Standards certified Beckman thermometer was used, with corrections for setting, certificate, and stem emergence, and the figure 3.63° signifies merely the elevation above the ice point determined just before and after the main experiment.

Product 1 was recrystallized without use of solvent, yielding product 4, a part of which was washed with concentrated sulfuric acid, yielding 5. Both 4 and 5 agreed exactly with 3 in melting point. In view of the constancy of the temperature, it was judged that the theoretical melting point (disappearance of last crystal) is not over 3.65°, and that this value ±0.03° is the melting point of pure dicyclohexyl. Apparently the figures of 4° (*4*) and "above 4°" (*7*) reported for this compound in the literature were not determined with special precision.

Presumably the impurities in any of the products so far encountered are not only of small amount, but of physical nature not entirely foreign to dicyclohexyl itself. Accordingly, the critical solution temperature values obtained with the five preparations are even more closely concordant than the melting points. Thus Nos. 1 and 2 are satisfactory for ordinary accuracy in estimation of water in alcohol.

Dicyclohexyl Point

PROCEDURE. To 2.0 cc. of the alcohol being tested, in a dry 15-mm. test tube, add 4.0 cc. of dicyclohexyl and stir with a dry thermometer. Heat until the mixture becomes a clear solution, and then allow to cool slowly, with continued stirring. As the critical solution temperature is approached, the liquid becomes opalescent, suggestive of very dilute soap solution. It is still clear enough so that the mercury thread in the immersed section of the thermometer is readily distinguished. Suddenly (within 0.2° temperature range) the liquid becomes completely turbid, and the mercury thread is no longer discernible even through as little as 5-mm. thickness of the liquid. The temperature at this stage (approximately the critical solution temperature) is recorded as the dicyclohexyl point, and the corresponding percentage of alcohol is read from the graph of Figure 2.

Acknowledgment

The author wishes to thank the Dow Chemical Company of Midland, Mich., and Ralph P. Perkins of that organization, for special materials and technical service furnished during this study.

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Unsaturation of Butadiene and Related Polymers

As Determined by Iodine Chloride Addition

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This paper describes procedures which have been developed to determine the unsaturation of various butadiene and related polymers and copolymers, as well as mixed vulcanizates of Buna S and rubber. These methods are based on the use of *p*-dichlorobenzene as a solvent and iodine chloride as the addition agent, following the general technique employed in the standard Kemp-Wijs method for the determination of the unsaturation of natural rubber.

The ratio of butadiene to styrene in copolymers has been calculated from the iodine value and from the carbon-hydrogen ratio; however, the accuracy of these procedures is subject to several variables which are discussed.

Unsaturation data are presented on highly purified emulsion-type polymers of butadiene-isoprene and butadiene-styrene which agree closely with the presence of one double bond for each diolefin molecule present. The reaction rate of Buna S with halogens is shown to agree closely to that of natural rubber hydrocarbon.

SINCE iodine chloride adds quantitatively to the double bonds in natural rubber hydrocarbon (*5*, *6*), it was considered important to study the reaction of various unsaturated synthetic elastomers with this reagent. Recently Cheyney and Kelley (*3*) found that the Wijs reagent reacted so slowly with polybutadiene and its copolymers swollen in chloroform or carbon disulfide, that the addition reaction required 48 hours or longer to approach completion. Butadiene and butadiene-styrene polymers are frequently only partially soluble in chloroform or carbon disulfide and, if soluble, they are partially precipitated by the addition of the glacial acetic acid in the Wijs reagent. This requires that the reaction proceed between the swollen polymer and the iodine chloride solution, which accounts in part for the long period needed to complete the reaction. Other complications, such as emulsion formation and occlusion of iodine in the precipitate during titration of the unreacted iodine chloride, are likely to be involved in the standard Kemp-Wijs procedure (*4*, *5*, *6*) which was developed for natural rubber.

The present investigation was undertaken to overcome these difficulties and to develop a rapid and accurate method which could be applied to various types of synthetic rubber-like polymers. It was also hoped that data obtained on the unsaturation of these polymers would throw some light on their chemical structure as related to polymerization procedure.

A study of numerous solvents showed that *p*-dichlorobenzene heated from 165° to 172° C. was the most satisfactory for general use. Cheyney and Kelley (*3*) objected to this solvent on the ground that cyclization of the synthetics takes place as the result of heating. The results of the present investigation, however, show that very little loss of unsaturation occurs in the polymers during the period of heating in *p*-dichlorobenzene necessary to attain a complete solution. The use of *p*-dichlorobenzene is also advantageous for use in the case of crude natural rubber, since solution is complete in less than one hour, which is important in the tougher and less soluble types. The present authors have confirmed the finding of Blake and Bruce (*1*) on the superiority of this solvent for vulcanized rubber as compared with tetrachloroethane (*5*). Soft vulcanized butadiene-styrene copolymers are also soluble in hot *p*-dichlorobenzene, making it possible to determine the total rubber content of a soft vulcanized mixture of natural and GR S rubber.

In order to avoid or substantially to reduce precipitation upon addition of Wijs solution, the iodine chloride was made up using carbon tetrachloride as a solvent in place of glacial acetic acid. In the case of the butadiene-nitrile copolymers the standard Wijs solution was employed, since the iodine chloride reaction product was more soluble in the presence of the glacial acetic acid.

Emulsions which formed during titration were broken by the addition of 25 cc. of alcohol. In the case of vulcanized GR S tire tread stocks containing channel black, it was found that by omitting the water and adding 75 cc. of alcohol, excellent results were obtained. In this case the presence of the larger quantity of alcohol caused the carbon black to settle rapidly, so the end point could be quickly obtained.

In the case of polybutadiene 0.10-gram samples gave low results, since the excess of iodine chloride is not sufficient to complete the reaction. Sample weights of 0.06 gram were found to give consistent results. In the case of the high-nitrile type of polymer, solution in *p*-dichlorobenzene is difficult; 20 to 60 passes through a clean tight mill before extraction greatly aided solution. This milling has been found to have no significant effect on the iodine value of any of the synthetics or of natural rubber and can be practiced to advantage whenever the polymer requires too long a period to dissolve.

General Recommended Procedure

Unless otherwise purified, samples are acetone-extracted in the standard manner in the absence of strong light for 16 hours. In the case of vulcanized samples this is followed by a 4-hour chloroform extraction. The extracted samples are freed from solvent by heating to constant weight in an oven at 70° C. under vacuum, cut into fine pieces (approximately 15-mesh), and preserved under nitrogen or in high vacuum prior to analysis to prevent oxidation.

The finely divided sample (0.10 gram, or 0.06 gram in case of polybutadiene) is placed in a 500-cc. Pyrex glass-stoppered vapor-release iodination flask with 50 grams of pure *p*-dichlorobenzene. The flask with contents is placed on a hot plate at a temperature of 175° to 185° C. with the vapor release on the iodine flask lined up properly to release the pressure that is formed during heating. It is desirable to cover the sides of the flask with an asbestos shield to prevent the solvent from solidifying in the upper part of the flask. The contents of the flask are gently whirled from time to time to facilitate solution, care being taken to avoid causing particles to adhere and scorch on the sides of flask above the solvent. The time for solution depends upon the nature of the polymer and usually varies from about 20 to 180 minutes. If the time required exceeds 3 hours and a rubber mill is available, it is recommended that the polymer be milled to increase its solution rate.

Following solution, the flask with its contents is removed and allowed to cool to room temperature. Before crystallization of

the p-dichlorobenzene is complete the partially solidified solution is liquefied by adding 50 cc. of chloroform. Twenty-five cubic centimeters of iodine chloride in carbon tetrachloride are added from a pipet, using a vacuum to suck the solution up uniformly and very slowly to avoid loss of iodine chloride by evaporation. A thin film of 15 per cent potassium iodide is placed on the stopper of the iodine flask just before closing and the solution allowed to stand for one hour at room temperature in the dark to complete the reaction. Twenty-five cubic centimeters of 15 per cent freshly prepared potassium iodide solution are added, followed by the addition of 50 cc. of distilled water. The excess iodine is immediately titrated with standard 0.1 N sodium thiosulfate solution, using freshly prepared starch indicator which is added towards the end of the titration. Twenty-five cubic centimeters of ethyl alcohol are added towards the end of the titration to break the emulsion.

Table I. Effect of Varying Experimental Conditions of Iodine Value of Polybutadiene

Solvents Employed	Polybutadiene	Heating Period in $C_6H_4Cl_2$	Iodine Chloride Solution Used	Time of Reaction	Iodine Value
	Gram	*Hours*		*Hours*	
75 cc. of CS_2	0.1040	...	Wijs	3	399.0[a]
	0.1034	...	Wijs	24	407.9[a]
75 cc. of $CHCl_3$	0.1031	...	Wijs	3	388.1[a]
	0.1026	...	Wijs	24	406.3[a]
50 grams of $C_6H_4Cl_2$ + 50 cc. of CS_2	0.1201	1.0	CCl_4	1	385.3
	0.1017	1.0	CCl_4	1	413.7
	0.1014	1.0	CCl_4	1	412.6
	0.0824	1.0	CCl_4	1	435.7
	0.0756	1.0	CCl_4	1	436.5
	0.0759	1.0	CCl_4	1	436.1
50 grams of $C_6H_4Cl_2$ + 50 cc. of $CHCl_3$	0.0753	1.0	CCl_4	1	435.0
50 grams of $C_6H_4Cl_2$ + 50 cc. of CS_2	0.0668	1.0	CCl_4	1	438.6
	0.0615	1.0	Wijs	1	409.1
	0.0613	6.0	CCl_4	1	435.9
	0.0604	1.0	CCl_4	1	438.5
	0.0606	1.0	CCl_4	1	440.9
	0.0511	1.0	CCl_4	1	440.1
	0.0510	1.0	CCl_4	1	440.5
	0.0313	1.0	CCl_4	1	441.7
	0.0221	1.0	CCl_4	1	440.4
	0.0218	1.0	CCl_4	1	441.8

[a] Heavy precipitation occurs upon adding Wijs reagent.

The end point is reached when the color change passes from a light brown to a light purple and finally to a colorless solution. When the end point is near, moderate shaking of the solution is necessary after each drop or partial drop of sodium thiosulfate is added. Violent shaking should be avoided to prevent breaking the flask. A blank is carried through all the operations of heating, etc. The difference in cubic centimeters of 0.1 N thiosulfate between the blank and the sample titration is used to calculate the iodine value:

$$\text{Iodine value} = \frac{\text{cc. of } 0.1\ N\ Na_2S_2O_3 \times 1.2692}{\text{wt. of sample in grams}}$$

Iodine Value of Polybutadiene

The effect of using different solvents on the iodine value of polybutadiene (German sodium Buna "85") under various conditions is shown by Table I. In the experiments where 0.1-gram samples are employed the iodine values are too low because the addition reaction is not complete. Some precipitation occurred in all cases where solution was effected by hot p-dichlorobenzene supplemented by carbon disulfide or chloroform and where iodine chloride in carbon tetrachloride was used. Where heavier precipitation occurred using Wijs solution, the increased time of reaction did not overcome the low results. Heating for 6 hours in p-dichlorobenzene resulted in about 1 per cent reduction in the iodine value. This shows that the effect on the unsaturation as the result of heating for 1 hour in p-dichlorobenzene can be neglected.

Since the excess iodine chloride appeared important, a series of determinations was carried out varying the sample weights. The samples were dissolved in 50 grams of boiling p-dichlorobenzene for 1 hour, 50 cc. of carbon disulfide and 25 cc. of 0.2 N iodine chloride in carbon tetrachloride were added, and the reaction was carried out for 1 hour at room temperature. The results are plotted in Figure 1 and show that a weight of sample of 0.05 to 0.06 gram is satisfactory. These weights correspond to an excess of iodine chloride of 50 to 60 per cent. About the same excess was found to be necessary to complete the iodine chloride reaction in 1 hour in the case of natural rubber (*6*).

To determine the tendency of polybutadiene to substitute, a series of determinations was carried out at room temperature and at 3° C. with variations in reaction periods from 1 to 24 hours. The sample weight was 0.06 gram and the procedure the same as the other series shown in Figure 1. Figure 1 indicates that substitution takes place slowly even at 3° C. A reaction period of 1 hour therefore appears to be justified, since the addition reaction apparently is completed in this period, provided the proper excess of reagent is employed. The high iodine values obtained by Cheyney and Kelley after a reaction period of 336 hours are undoubtedly due in part to substitution.

The theoretical iodine value for polybutadiene is 469.6. The acetone-extracted sodium butadiene polymer was not a pure hydrocarbon as judged from its combustion analysis. The nonhydrocarbon portion appears to be made up largely of ash and combined oxygen.

Two butadiene and one isoprene emulsion polymers were prepared under the supervision of B. S. Biggs of these laboratories. The latex was coagulated by pouring it into an excess of alcohol and the coagulum was washed in warm 50 per cent alcohol and finally in pure alcohol. It was finally dried to

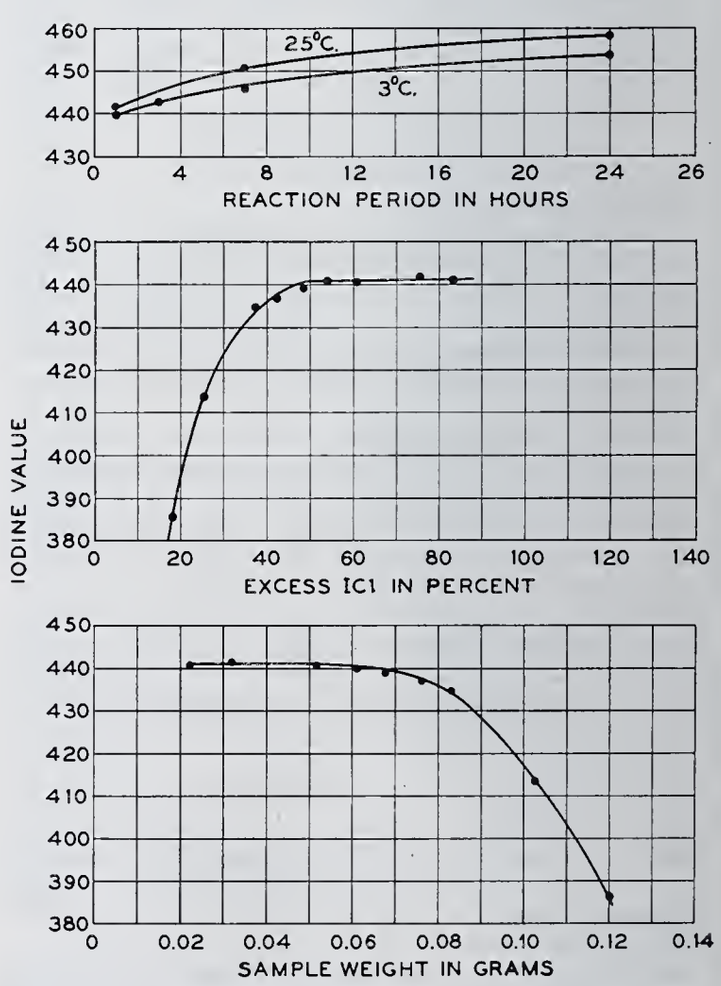

Figure 1. Iodine Value of Polybutadiene

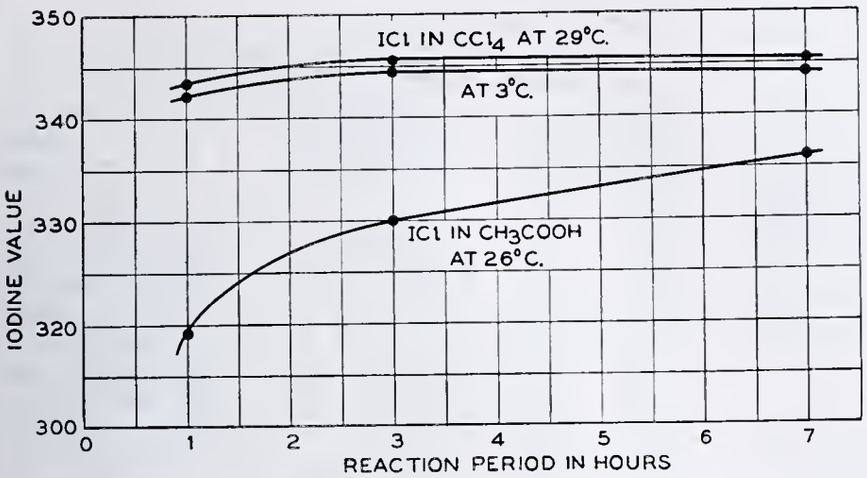

FIGURE 2. IODINE VALUE OF BUTADIENE-STYRENE COPOLYMERS

TABLE II. COMBUSTION ANALYSES AND IODINE VALUES OF BUTADIENE AND ISOPRENE POLYMERS

Polymer	Carbon	Hydrogen	C/H Ratio	Iodine Value	Theoretical Iodine Value
	%	%			%
Polybutadiene (German Na 85)[a]	88.08	10.87	8.10	440.5	93.7
Polybutadiene (emulsion method)	88.86	11.21	7.93	455.1	96.6
Polyisoprene (emulsion method)	...	...	..	369.1	99.0

[a] Acetone extracted before analysis.

constant weight under high vacuum at 60° C. A highly purified lot of isoprene was also polymerized by the soap emulsion method.

The iodine values and analysis of these polymers are given in Table II in comparison with the acetone-extracted German Buna "85" viscosity polymer made by mass polymerization, using sodium as a catalyst. Whereas the German sodium polymer of butadiene has an iodine value somewhat below the theoretical value, the unsaturation of the emulsion polymers agreed fairly closely with theory.

The iodine value of 440 for the extracted sodium Buna "85" is lower than theory, which is 469.6, even when the nonhydrocarbon portion is considered. The unsaturation of sodium Buna "85" is calculated to be 93.69 per cent of theory. This low unsaturation value, together with its low solubility and plasticity, may be taken as evidence of the presence of some type of interlinkage between the polymer chains; however, knowledge of these structural details is lacking. Chain branching, oxygen bridging, or cyclization during polymerization has been suggested to account for the differences in the properties of butadiene and isoprene polymers as compared with natural rubber.

Iodine Value of Butadiene-Styrene Copolymers

The effect of using different solvents and experimental conditions on the iodine value of an acetone-extracted 75/25 butadiene-styrene copolymer is shown in Table III. These results indicate that the use of either chloroform or carbon disulfide to supplement the *p*-dichlorobenzene is satisfactory, provided iodine chloride in carbon tetrachloride is employed in place of Wijs solution. Chloroform, however, is preferred over carbon disulfide because of the objectionable odor and fire hazard of the latter solvent.

Data from Table II, plotted in Figure 2, show evidence of very little substitution in the case of the butadiene-styrene copolymer. Reaction conditions of 1 hour at room temperature appear to be a satisfactory selection when iodine chloride in carbon tetrachloride is employed. The use of Wijs solution gives low results even after a 48-hour reaction period. Under these conditions the iodine values obtained are in agreement with those of Cheyney and Kelley (*3*). The data in Figure 2 show that Wijs solution is unsatisfactory for use with butadiene-styrene copolymers, whereas iodine chloride in carbon tetrachloride appears to meet the necessary requirements. Iodine bromide (Hanus solution) was also tried but was found to be slower than Wijs solution under identical conditions. After 7 hours' reaction period, the Hanus value was 318, in contrast with 336 for the Wijs value for the same period.

Reactivity of Double Bonds in Buna S

Since some of the double bonds in Buna S appear to be located in side-chain vinyl groups as a result of 1,2 addition polymerization of the butadiene, it was thought that this might affect their reactivity as compared with the double bonds in the main chain resulting from 1,4 addition. In order to detect such a difference, the reactivity of Buna S with iodine, iodine chloride, and iodine bromide was compared with rubber under identical conditions. These experiments are outlined in Table IV and the data show that very little difference exists between the reactivity of Buna S and rubber hydrocarbons towards iodine, iodine chloride, or iodine bromide.

Determination of Styrene in Butadiene-Styrene Copolymers

Since no method was available for the determination of the styrene content of GR S rubber or other butadiene-styrene

TABLE III. IODINE NUMBER OF A BUTADIENE-STYRENE COPOLYMER UNDER VARIOUS EXPERIMENTAL CONDITIONS

Solvents Employed	Butadiene-Styrene Copolymer	Heating Period in $C_6H_4Cl_2$	Iodine Chloride Solution Used	Temp. of Reaction	Time of Reaction	Iodine Value
	Gram	*Hours*		*° C.*	*Hours*	
75 cc. of $CHCl_3$	0.10	...	Wijs	20–30	48	334.1[a]
75 cc. of CS_2	0.10	...	Wijs	20–30	48	330.0[a]
50 grams of $C_6H_4Cl_2$ + 50 cc. of CS_2	0.10	1.0	Wijs	26	1	319.3
	0.10	1.0	Wijs	26	3	330.2
	0.10	1.0	Wijs	26	7	336.0
50 grams of $C_6H_4Cl_2$ + 50 cc. of $CHCl_3$	0.10	1.0	Wijs	26	7	335.8
50 grams of $C_6H_4Cl_2$ + 50 cc. of CS_2	0.05	1.0	CCl_4	29	1	343.5
	0.10	1.0	CCl_4	29	1	343.5
50 grams of $C_6H_4Cl_2$ + 50 cc. of $CHCl_3$	0.10	1.0	CCl_4	29	1	344.0
50 grams of $C_6H_4Cl_2$ + 50 cc. CS_2	0.10	6.0	CCl_4	29	1	343.2
	0.10	1.0	CCl_4	29	3	345.4
	0.10	1.0	CCl_4	29	7	345.5
	0.10	1.0	CCl_4	3	1	342.2
	0.10	1.0	CCl_4	3	3	344.0
	0.10	1.0	CCl_4	3	7	343.8

[a] Solid phase present throughout reaction.

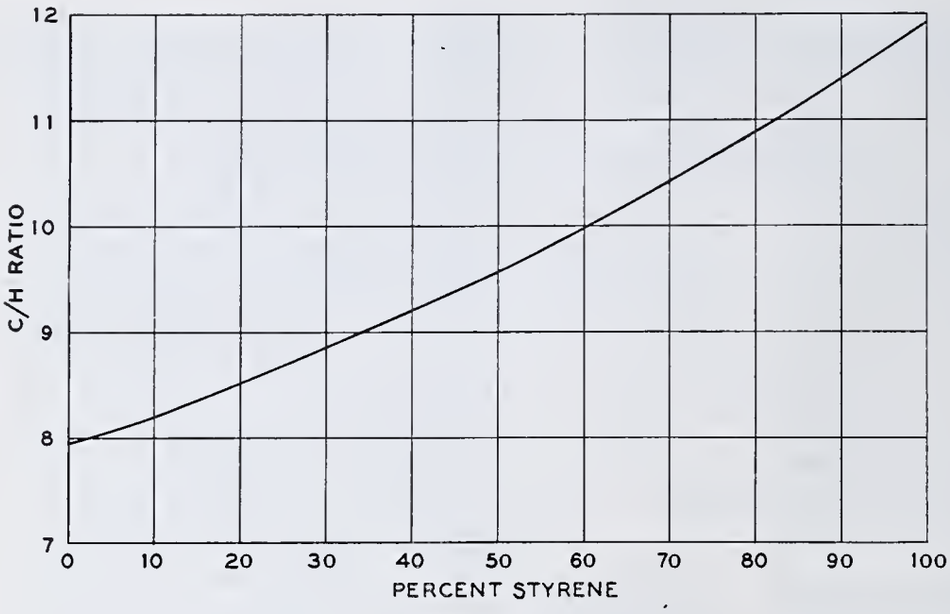

FIGURE 3

TABLE IV. RELATIVE REACTIVITY TOWARDS HALOGENS OF BUNA S AND RUBBER[a]

Polymer	0.2 *N* Addition Agent Added	Reaction Period	Reagent Consumed	Addition Agent Consumed
		Min.	*Cc.*	%
Rubber	25 cc. of I_2 in CCl_4	240	0.70	2.8
Buna S	25 cc. of I_2 in CCl_4	240	0.56	2.3
Rubber	11 cc. of ICl in CCl_4	10	10.56	96.0
Buna S	11 cc. of ICl in CCl_4	10	10.00	91.0
Rubber	5 cc. of ICl in CCl_4	10	4.90	98.0
Buna S	5 cc. of ICl in CCl_4	10	4.88	97.5
Rubber	3 cc. of ICl in CCl_4	60	2.94	98.0
Buna S	3 cc. of ICl in CCl_4	60	2.97	99.0
Rubber	3 cc. of IBr in CCl_4	10	1.90	63.2
Buna S	3 cc. of IBr in CCl_4	10	1.90	63.2

[a] 0.1000 gram of acetone-extracted crepe or Buna S dissolved in 75 cc. of chloroform and reaction carried out in dark at 25° C.

copolymers, the present authors employed combustion analyses, using the carbon-hydrogen ratio to calculate the styrene content. As the styrene content increases from 0 to 100 per cent the carbon-hydrogen ratio will vary from 7.943 to 11.915, as shown in Figure 3. A variation of ±0.2 per cent in carbon will change the carbon-hydrogen ratio in a 21 per cent styrene–79 per cent butadiene copolymer by ±0.017, which is equivalent to ±0.5 per cent styrene. A variation of only 0.023 per cent in the hydrogen content will be equivalent to the same change in carbon-hydrogen ratio and styrene content; therefore, special precautions must be taken in the combustion analyses to avoid errors. The present authors depended upon a carefully conducted micromethod combustion. These analyses were carried out by F. C. Koch of these laboratories. In the case of soap-free polymers, the accuracy is believed to be sufficient to establish the styrene content to within 1 or 2 per cent.

Data giving the variation of iodine value and per cent of theoretical unsaturation are shown in Table V and Figure 4 for copolymers of butadiene and styrene of different styrene contents. The largest group analyzed was the regular commercial polymer made using charging ratios of 25 styrene and 75 butadiene. In all cases the determined styrene content was found to be somewhat less than the charging ratio. The values for polybutadiene in Figure 4 are those found for the sodium "85" viscosity polymer and for a purified benzene-soluble polybutadiene made by the emulsion process. Data on the latter polymer are given in Table III.

It is to be noted from Table V and Figure 4 that the iodine values of butadiene-styrene copolymers are approximately proportional to their styrene content. The iodine value can therefore be employed to calculate the styrene content. However, variations in the amount of unsaturation lost during polymerization and the presence of soap and other impurities will influence the accuracy of such a procedure. The commercial emulsion polymers contain variable amounts of inorganic matter, their ash contents usually varying from 0.3 to 1.2 per cent. Combustion analyses indicate that they contain some combined oxygen, in some cases possibly as much as 0.2 to 0.5 per cent.

The data in Table V on purified polymers are evidence that there is very little loss in unsaturation in the butadiene beyond the one double bond per butadiene molecule which would result from linear chain polymerization. The data on the per cent of theoretical unsaturation for the butadiene present in the acetone-extracted commercial polymers are undoubtedly influenced somewhat by errors in the results for styrene content based on microcombustion analyses. These data indicate that 2 to 10 per cent of the theoretical unsaturation of the butadiene after one of its double bonds enters the chain is used up during the polymerization process. However, it was found, as shown in Table V, that by careful fractionation of a benzene solution of a soap-free benzene-soluble type polymer the unsaturations of the higher molecular fractions are close to the theoretical value. The whole polymer, No. 12 in Table V, was unsaturated to 98.3 per cent of theory. In this case the styrene content was determined by an interferometer method recently developed by W. O. Baker and

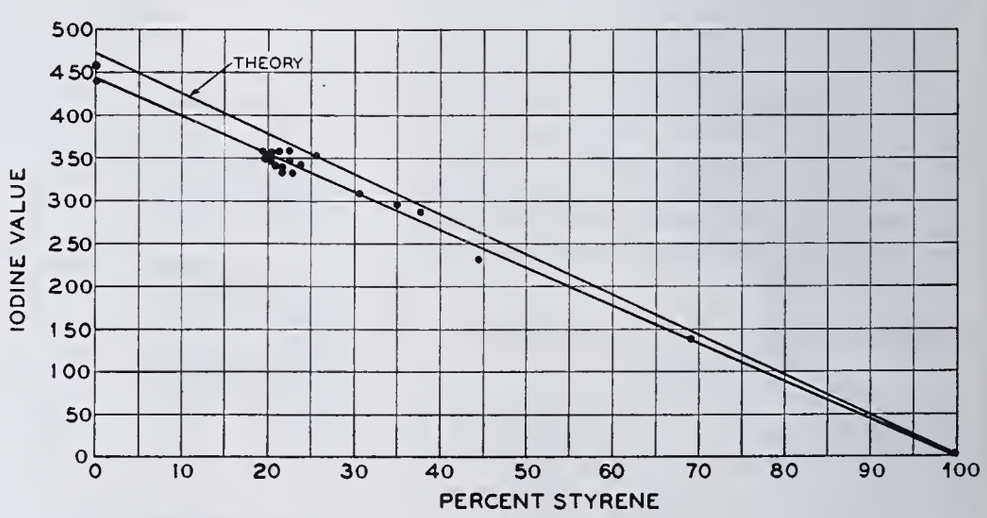

FIGURE 4

Table V. Carbon-Hydrogen Ratios, Calculated Styrene Contents, and Iodine Values of Butadiene-Styrene Polymers

Polymer	Carbon	Hydrogen	C/H Ratio	Styrene from C/H	Iodine Value	Styrene from I. V.[a]	Theoretical Unsaturation[b]
	%	%		%		%	%
1	89.00	10.39	8.56	21.0	360.0	23.4	97.0
2	88.28	10.34	8.54	20.8	342.0	27.2	91.9
3	88.65	10.44	8.50	19.0	357.8	23.9	94.0
4	89.00	10.36	8.59	22.0	338.2	28.0	92.3
5	89.04	10.40	8.56	21.0	341.2	27.4	92.0
6	89.70	10.42	8.61	22.5	334.0	28.9	91.8
7	88.08	10.36	8.50	19.0	352.8	24.9	92.7
8	88.85	10.37	8.58	21.6	342.0	27.2	92.9
9	88.35	10.31	8.56	21.0	343.1	27.0	92.2
10	88.38	10.28	8.60	22.2	340.0	27.6	93.0
11	89.09	10.32	8.63	23.2	343.5	26.9	95.2
12	88.52	10.29	8.61	22.7	356.7	24.1	98.3
13	89.52	10.13	8.84	29.6	313.4	33.4	94.8
14	89.27	9.83	9.08	36.4	291.1	38.1	97.4
15	89.47	9.59	9.33	44.0	234.0	50.0	89.0
16	89.80	8.81	10.19	66.0	142.4	69.8	89.2
Purified Polymers							
P-1[c]	89.24	10.28	8.68	25.0	353.5	24.8	100.4
P-2[c]	89.48	10.23	8.73	26.3	346.5	26.3	100.1
P-3[c]	89.34	10.25	8.72	25.8	353.3	24.8	101.3
P-4[d]	89.80	10.42	8.62	23.0	356.1	24.2	98.5
P-5[d]	89.87	10.32	8.61	22.7	359.3	23.6	98.7
P-6[d]	89.33	10.35	8.63	23.2	359.1	23.6	99.5
P-7[d]	89.26	10.25	8.70	25.3	359.8	23.5	100.3
P-8[d]	89.27	10.29	8.67	24.5	359.6	23.5	100.1
P-9[d]	88.76	10.35	8.58	21.9	355.5	24.4	97.0
P-10[d]	88.06	10.30	8.55	20.7	352.1	25.0	94.8

[a] $\% \text{ styrene} = 100 - \left(\frac{\text{iodine value}}{469.6} \times 100\right)$.

[b] Calculated by employing 469.6 as the theoretical iodine value for polybutadiene—i. e., $\frac{\text{iodine value of polymer} \times 100}{\text{wt. proportion of butadiene in polymer} \times 469.6} = \%$ of theoretical unsaturation.

[c] Prepared by coagulation of polymer emulsion with alcohol and washing with alcohol. Addition of antioxidant was omitted.

[d] Prepared from benzene-soluble soap-free commercial polymer No. 12 by fractional coagulation from dilute benzene solution upon addition of methyl alcohol. These fractions represent about 90 per cent of the polymer and are given in order of decreasing molecular weights.

J. H. Heiss of these laboratories, giving a styrene content of 23.4 per cent and a theoretical unsaturation of 99.1 per cent.

In the case of benzene-soluble emulsion-type polymers purified by coagulation and washing with ethyl alcohol, the data in Table V show that very little loss in the unsaturation of butadiene beyond one of its double bonds occurs during polymerization. In this case the styrene contents given were also checked by the above-mentioned interferometer method. The iodine values of the alcohol precipitated and purified emulsion polymers from pure butadiene and specially purified isoprene also showed close agreement with theory.

Since soap is not removed by acetone extraction, its presence in the polymer will reduce the iodine value. The soap content is generally less than 1 per cent, but in some cases more may be present. In a special case where a polymer was found to contain 5.25 per cent soluble soap calculated as sodium stearate the iodine value of the acetone-extracted residue was found to be 345.1. When the acetone-extracted polymer was refluxed with a mixture of 2 volumes of benzene and 1 of alcohol for 1 hour to remove this soap, the iodine value of the polymer increased to 350.6. This is a lower value than expected, which can only be accounted for on the basis that the soap acids were unsaturated.

The presence of soap will lower the carbon-hydrogen ratio, resulting in calculated styrene contents which are too low. Consequently the calculated per cent of theoretical unsaturation will be too low. The presence of soap and other impurities will reduce the iodine value, which also results in a lower calculated theoretical unsaturation.

When the styrene content is calculated from the iodine value as shown in Table III, it is seen that the presence of impurities giving a low iodine value will result in a calculated styrene content which is too high. This method for obtaining the styrene content will also give too high results if the butadiene present does not have theoretical unsaturation. If care is taken to remove the soap, the iodine value method should prove to be a fairly accurate procedure for determining the styrene content of commercial GR S.

Iodine Value of Butadiene–Acrylic Nitrile Polymers

The effect of different reaction conditions on the iodine value of butadiene–acrylic nitrile copolymer is shown in Table VI. These data show that either of two procedures is satisfactory. In one procedure the polymer can be passed 60 times through a tight mill roll and rendered soluble in chloroform, thereby avoiding the use of *p*-dichlorobenzene. However, to save time a lesser amount of milling together with the use of *p*-dichlorobenzene will be found advantageous. In either case the use of Wijs solution is preferred, along with addition of 50 cc. of chloroform to the *p*-dichlorobenzene solution. The polar nature of the glacial acetic acid increases the solubility of the polymer and its iodine chloride addition product.

The results of chemical analysis and iodine value of these polymers are given in Table VII, together with butadiene contents calculated from both carbon-hydrogen ratios and nitrogen contents. The loss in unsaturation upon polymerization is higher than in the case of the butadiene-styrene polymers. This is also reflected in the low plasticity and difficulty in processing the nitrile copolymers, since cross-linking or cyclization reactions during polymerization would be expected to result in loss of unsaturation and decreased processibility.

Iodine Value of Polychloroprene

Tests made on GN neoprene using the present method showed that in 2 hours at room temperature the reaction had proceeded to 56.9 per cent completion (iodine value 169.1)

Table VI. Iodine Value of Butadiene-Nitrile Copolymers under Various Experimental Conditions

Solvents Employed	Butadiene-Nitrile Copolymers	No. of Passes through Mill	Heating Period in $C_6H_4Cl_2$	Iodine Chloride Solution Used	Time of Reaction	Iodine Value[a]
	Gram		*Hours*		*Hours*	
	(Copolymer containing 6.54% nitrogen)					
50 grams of $C_6H_4Cl_2$ + 50 cc. of CS_2	0.1	0	2.5	Wijs	1	315.3[b]
50 grams of $C_6H_4Cl_2$ + 50 cc. of $CHCl_3$	0.1	0	2.5	CCl_4	1	306.0[b]
	0.1	20	1.25	Wijs	1	302.1 (303.0)
	0.1	0	2.5	Wijs	1	300.0 (302.5)
50 grams of $C_6H_4Cl_2$ + 75 cc. of $CHCl_3$	0.1	0	2.5	Wijs	1	302.2
50 grams of $C_6H_4Cl_2$ + 50 cc. of $CHCl_3$	0.1	0	2.5	Wijs	3	300.5
	0.1	0	2.5	Wijs	7	306.0 (307.2)
	0.05	0	2.5	Wijs	7	306.2
	0.1	0	4.5	Wijs	1	300.1
50 grams of $C_6H_4Cl_2$ + 75 cc. of $CHCl_3$	0.1	0	4.5	Wijs	1	300.4
50 grams $C_6H_4Cl_2$ + 50 cc. of $CHCl_3$	0.1	0	9	Wijs	3	239.0 (245.3)[c]
	0.1	20	3	Wijs	3	264.8 (263.8)
	0.1	60	2	Wijs	3	262.0
	0.1	60	1	Wijs	3	265.8
	0.05	60	1	Wijs	3	265.5
75 cc. of $CHCl_3$	0.05	60	..	Wijs	3	264.2
	0.05	60	..	Wijs	30	268.2

[a] Reaction carried out at room temperature.
[b] Heavy ppt. formed.
[c] Polymer undissolved.

Table VII. Analyses of Butadiene–Acrylic Nitrile Polymers

	Polymers	
	Regular nitrile content	High nitrile content
Carbon, per cent	81.53	79.80
Hydrogen, per cent	9.53	8.90
C/H ratio	8.55	8.97
Butadiene content from C/H (A), per cent	79.2	66.5
Nitrogen, per cent	6.54	10.19
Acrylic nitrile, per cent	24.8	38.5
Butadiene from N (B), per cent	75.2	61.5
Iodine value	302.2	266
Theoretical iodine value from A, per cent	81.0	85.0
Theoretical iodine value from B, per cent	86.0	92.3
Average % theoretical iodine value from A and B	83.5	88.6

and to 64.6 per cent (iodine value 191.9) after a 24-hour reaction period. The theoretical iodine value for polychloroprene is 297.1. As no difficulties were experienced in the procedure, it is concluded that the chlorine atom attached to the carbon atom in the second position in the butadiene base unit offers hindrance to the iodine chloride addition reaction. This was previously noted (*6*), when it was found that addition of iodine chloride by the Kemp-Wijs method took place to the extent of only 30 per cent. A comparison of these results also shows the greater activity of the iodine chloride in carbon tetrachloride. Another experiment employing the present procedure using 0.05 gram of neoprene and a 24-hour reaction period at room temperature resulted in forcing the addition to 67.3 per cent, corresponding to an iodine value of 199.9.

Iodine Value of Butyl Rubber

The present method, or the Kemp-Wijs procedure, was found not to be applicable to butyl rubber, since substitution occurs so readily in both procedures as to introduce large uncertainties in the low unsaturation values. Various procedures have been studied (Table VIII).

Samples of 0.5 gram were used in all cases. In the case of chloroform only as the solvent, it required overnight to dissolve the butyl rubber. When hot *p*-dichlorobenzene was employed, as in the case of the butadiene polymers, the solution time ranged from 20 to 30 minutes. The concentration of sodium thiosulfate solution was reduced to 0.05 *N* to increase the accuracy of the titration. In other respects the procedure was the same as previously outlined.

The best choice of analytical procedure appears to be to employ 50 grams of *p*-dichlorobenzene and 50 cc. of chloroform as a solvent to obtain maximum speed. The use of 5 cc. of Wijs solution and carrying out the reaction for 2 hours in the dark at ice-water temperature appear to keep substitution at a minimum, and at the same time complete the addition reaction with a series of butyl rubbers of varying unsaturation.

It is seen that a sample of polyisobutylene having an average molecular weight of about 30,000 has an iodine value of 1.36, corresponding to an unsaturation of 0.36 per cent. If this unsaturation arises from free end valences, the unsaturation of the butyl rubber arising from the addition of the diolefin should be corrected by subtracting this value. On the other hand, polyisobutylene may contain some diolefin.

Hanus solution and iodine chloride in carbon tetrachloride were tried but were found to be more reactive than Wijs solution, causing considerable substitution. In the case of a 1.45 butyl rubber, the iodine values at room temperature and 1-hour reaction period in the dark were: Hanus, 6.86; iodine chloride in carbon tetrachloride, 6.57; and Wijs, 4.25.

Estimation of Natural and Synthetic Rubber in Mixed Vulcanizates

The chromic acid oxidation method of Kheraskova and Korsunskaya (*7*) which was improved by Burger, Donaldson, and Baty (*2*) serves to determine the natural rubber content of a mixture of GR S and natural rubber. This method, however, will not give the combined amount of natural and GR S rubber in an unknown vulcanized mixture. For this purpose the authors have applied the present method to finely divided samples which were first acetone- and chloroform-extracted in the regular manner.

The composition tested was a tread formula given in Table IX. The results of analysis presented in this table appear to be satisfactory.

Duplicability of Method

The duplicability of the present iodine chloride method is indicated by Table X, which shows that the present procedure as applied either to crepe or butadiene-styrene copolymers is probably duplicable within ±0.25 per cent. Care must be taken, however, to guard against nonhomogeneity in the

Table VIII. Iodine Number and Isoprene Content of Butyl Rubber and Polyisobutylene under Various Experimental Conditions

Polymer	Solvents Used	Iodine Chloride Solution Used, Wijs	Reaction Period	Reaction Temp.	Iodine Value	Unsaturation[a]
		Cc.	*Hours*	*° C.*		*%*
Butyl B-1.45	100 cc. of $CHCl_3$	20	0.5	24	5.37	1.44
		20	1	24	6.00	1.61
		20	4	24	7.10	1.90
		10	0.5	24	4.90	1.31
		10	1	24	5.49	1.47
		10	4	24	6.65	1.78
		5	0.5	24	4.13	1.11
		5	1	24	4.50	1.21
		5	4	24	5.62	1.51
		2	0.5	24	3.22	0.86
		2	1	24	3.22	0.86
		2	4	24	3.48	0.93
		10	0.5	3	3.87	1.04
		10	1	3	4.24	1.14
		10	4	3	4.88	1.31
		5	0.5	3	3.36	0.90
		5	1	3	3.44	0.92
		5	4	3	3.86	1.04
		3	0.5	3	3.29	0.88
		3	1	3	3.32	0.89
		3	4	3	3.49	0.94
		2	0.5	3	3.17	0.85
		2	1	3	3.22	0.86
		2	4	3	3.17	0.85
Polyisobutylene, 30,000 molecular weight		20	1	24	1.37	0.37
		10	1	3	1.37	0.37
		2	0.5	3	1.39	0.37
		2	1	3	1.37	0.37
		2	4	3	1.39	0.37
	50 grams of $C_6H_4Cl_2$ + 50 cc. of $CHCl_3$	5	0.25	3	1.09	0.30
		5	1	3	1.15	0.31
		5	2	3	1.36	0.36
		5	3	3	1.36	0.36
Butyl X		5	0.25	3	3.00	0.81
		5	1	3	3.20	0.86
		5	2	3	3.32	0.89
		5	3	3	3.32	0.89
Butyl X-1		5	0.25	3	4.58	1.23
		5	1	3	5.16	1.38
		5	2	3	5.36	1.44
		5	3	3	5.46	1.46
Butyl X-2		5	0.25	3	6.50	1.74
		5	1	3	7.35	1.97
		5	2	3	7.80	2.09
		5	3	3	7.91	2.12

[a] $\text{Unsaturation} = \frac{\text{iodine number of polymer} \times 100}{372.8}$.

Table IX. Analysis of Natural and GR S Vulcanizates

	I	II	III
Rubber Tread Compositions[a]			
GR S (21% styrene)	100.00	50.00	25.00
Smoked sheets	....	50.00	75.00
Stearic acid	3.00	3.00	3.00
Kosmobile 77	45.00	45.00	45.00
Zinc oxide	5.00	5.00	5.00
Sulfur	3.00	3.00	3.00
Santocure	1.25	1.25	1.25
El. Sixty	0.50	0.50	0.50
Total	157.75	157.75	157.75
Calculated Rubber Contents			
Natural rubber, per cent	0.00	31.8	47.6
Natural rubber hydrocarbon (% natural rubber × 0.95), per cent	0.00	30.2	45.2
GR S rubber, per cent	63.4	31.7	15.8
GR S rubber hydrocarbon (% GR S × 0.925), per cent	58.7	29.3	14.7
Chemical Analysis			
Acetone extract, per cent	7.8	5.6	4.0
Chloroform extract, per cent	2.0	1.0	0.75
Sulfur, combined, per cent	1.27	1.86	2.13
Iodine value	225.1	232.6	235.2
Natural R. H. (chromic acid method), per cent	1.45	31.3	45.3
Natural rubber, per cent	1.53	33.0	47.6
GR S hydrocarbon, per cent	58.8	29.0	15.9
GR S rubber (GR S hydrocarbon ÷ 0.93), per cent	63.1	31.2	17.1

$$\% \text{ GRS hydrocarbon} = \frac{A(100\text{-}B) - 372.8\,C}{D} \times 100$$

A = iodine value of extracted vulcanizate
B = combined per cent acetone and chloroform extracts
C = per cent natural rubber hydrocarbon. Determined by chromic acid method
D = iodine value of extracted GR S. Average for 13 lots GR S rubber = 347.0

[a] Vulcanized 20 minutes at 142° C.

samples. Errors in sample weight should be kept within ±0.0001 gram and the titration within ±0.03 cc. Since the end point in the titration is sensitive to 0.01 cc. it is not difficult to duplicate results within the above limits.

Substitution takes place more readily with the new method than with the Kemp-Wijs procedure, owing to the greater chemical activity of the iodine chloride in carbon tetrachloride as compared with the Wijs reagent. By limiting the time of reaction to 1 hour, the error due to substitution is very small and as judged from the results on crepe rubber the addition reaction appears to be completed in this time.

Table X. Iodine Value of Crepe and Butadiene-Styrene Copolymer Rubber under Various Conditions

Polymer	Method	Reaction Time *Hours*	Reaction Temp. *° C.*	Iodine Value	Difference %
A	Present	1	24	356.1	0.34
		1	24	357.3	
B	Present	1	24	312.7	0.49
				314.2	
C	Present	1	24	296.6	0.27
				297.4	
Crepe rubber	Present	1	24	352.3	0.09
Crepe rubber[a]	Present	1	24	352.0	
Crepe rubber	Present	16	24	373.0	
Crepe rubber[a]	Kemp-Wijs	2	3	352.5	
Crepe rubber[a]	Kemp-Wijs	1	24	353.9	

[a] 12 passes through tight mill rolls.

Conclusion

The procedures outlined in this paper make it possible to determine accurately the unsaturation of a wide range of synthetic rubberlike substances prepared by the polymerization of mono- and diolefins.

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Preparation of Phosphomolybdic Acid from Phosphoric Acid and Pure Molybdic Acid

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METHODS of preparation of phosphomolybdic acid appearing in the literature are intricate and indirect, usually involving several intermediate steps (*2*). This reagent can, however, be directly prepared from pure molybdic oxide and phosphoric acid (*1*). The molybdic oxide used by the author contained not more than 0.05 per cent impurities.

The method given below saves both time and materials in this preparation. It is applicable also to the preparation of phosphotungstic acid by substitution of tungstic for molybdic oxide. By changing the molecular proportions, any one of the heteropoly acids of phosphorus and molybdenum can be produced. The same holds true for tungsten.

Pure molybdic oxide (144 grams, 1 mole) is weighed out and to it are added 9.61 grams (1/12 mole) of 85 per cent phosphoric acid (U. S. P.) and enough water to make a total volume of approximately 1.5 liters. On stirring, the suspension is white, having the appearance of milk. It is placed on a hot plate and heated to boiling. As the temperature increases the color changes from white to a canary yellow. After boiling for approximately 3 hours, some molybdic oxide still remains in suspension. It is allowed to settle 2 minutes and filtered on an 11-cm. No. 52 Whatman filter paper. The residue is washed with 50 cc. of distilled water. The dried residue weighs 27.6 grams, so that only 116.4 grams of molybdic oxide have gone into solution. The filtrate is clear and deep yellow in color. It is evaporated in a 1500-cc. beaker on the hot plate (requiring about 3 hours) to a volume of approximately 100 cc. The solution at this point is deep orange in color and boils at 106° C. It is then removed from the hot plate and allowed to cool to room temperature overnight. The crystals which form occupy almost the entire volume, leaving only a small amount of supernatant liquid. The crystals are filtered without washing and dried somewhat on a 9-cm. No. 41-H Whatman filter paper.

The filtrate has a volume of 18 cc. and contains some 10 to 15 grams of phosphomolybdic acid. The damp crystals weigh 130 grams when dried at room temperature in a vacuum, and the final weight is 111.7 grams. The product is deep yellow to orange in color and has lost some of its crystalline appearance.

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Improved Electrometric Apparatus

For Use with the Karl Fischer Method for Determination of Water

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WATER is one of the materials most frequently determined in analytical laboratories; the control of many plant operations and the evaluation of many research data are dependent on a knowledge of the water content of the products and intermediates concerned.

One of the best of the numerous methods so far developed, first proposed by Fischer in 1935 (*2*), involves the direct determination of water by titration with a solution of iodine, sulfur dioxide, and pyridine in methanol. Since that time, Smith, Bryant, and Mitchell (*5*) have studied the nature of the reaction and established the proper stoichiometric relationship of the various constituents of the reagent and their relation to the water removed. In their work the end point was determined visually—i. e., taken at the point where the color of the solution being titrated changed from pale yellow to a dark brown, owing to a small excess of the reagent.

For dark-colored samples, Almy, Griffin, and Wilcox (*1*) developed a potentiometric method for determining the end point by measuring the potential difference of a platinum-tungsten electrode system. Although this method gave good results, the laboratory model Beckman pH meter used for measuring the electromotive force is expensive and the change of e. m. f. at the end point is comparatively small, only about 20 millivolts.

Wernimont and Hopkinson (*6*) recently utilized the "dead-stop" end-point method of Foulk and Bawden (*3*) and pointed out that this method gives sharper and more reproducible end points than either the visual or the potentiometric method. The dead-stop method is based on the fact that when an electromotive force of about 10 millivolts is impressed upon two platinum electrodes immersed in Karl Fischer reagent, current flows so that the galvanometer is deflected off the scale. During the titration the galvanometer remains deflected until at the end point it suddenly comes to zero reading. The work of these authors was duplicated in this laboratory with excellent success.

Apparatus and Reagents

This paper describes a simpler, less expensive electrometric apparatus using the dead-stop technique for determining the end point. The parts can be procured for about ten dollars. The assembly is rugged and is suitable for use in either research or plant control laboratories.

In this apparatus the rather delicate and expensive galvanometer used by Wernimont and Hopkinson (*6*) is replaced by a cathode ray "magic eye" tube of the type used in a great number of laboratory instruments. The circuit used is essentially the titrimeter circuit of Serfass (*4*), modified and adapted to the determination of the Karl Fischer end point. Two platinum electrodes with a small polarizing voltage are placed in the solution being titrated. The electrical changes on these electrodes are amplified by a 6F5 vacuum tube. The amplifier is coupled to the 6E5 cathode ray tube which serves as the indicator.

The power for this apparatus is furnished by the 110-volt alternating current lines through a 6H6 tube acting as a rectifier-doubler. The original Serfass circuit did not use the rectifier as a doubler, thus allowing his titrimeter to be used on both alternating and direct current supply lines. The authors' rectifier-doubler circuit gives approximately a 250-volt direct current supply for the operation of the amplifier and cathode ray tubes. Although it cannot be used on direct current supply lines, this circuit has the advantage of giving a much brighter fluorescence on the target of the 6E5 cathode ray tube. The proper setting of the sensitivity control, a control for varying the bias of the grid of the 6F5 tube, was found in all cases to be the position of minimum resistance; therefore, this control was omitted from the finished instrument. Since it is necessary to use a polarizing voltage at all times, no provision is made for disconnecting this polarizing voltage.

The polarizing voltage is controlled by the current flowing through the 6F5 tube and varies with this current. At the beginning of the titration, this voltage is less than 1 millivolt and increases to about 15 millivolts at the end point. The current flowing between the electrodes during the titration is about 5 microamperes and becomes practically zero at the end point.

It was found that fluctuations of 5 volts or less in the line voltage do not have an appreciable effect on the end point. Such fluctuations do cause small changes in the eye position, but the end point is still readily discernible. If the supply voltage is unstable, a constant-voltage transformer should be used or a voltage regulator tube should be incorporated into the circuit.

The electrometric assembly is wired in accordance with Figure 1 and is mounted in a small metal cabinet. The complete setup is shown in Figures 2 and 3.

Titrations are made by adding an excess of Karl Fischer reagent and back-titrating the excess with a standard solution of water in methanol. The direct titration of the sample with Karl Fischer reagent was tried but was found to give premature and fading end points. It was decided, therefore, to adopt the back-titration with standard methanol. The titration flask containing the sample with excess Karl Fischer reagent is placed in position beneath the standard methanol buret. The eye control is turned carefully to the point where the "magic eye" is completely open. The eye control must not be turned

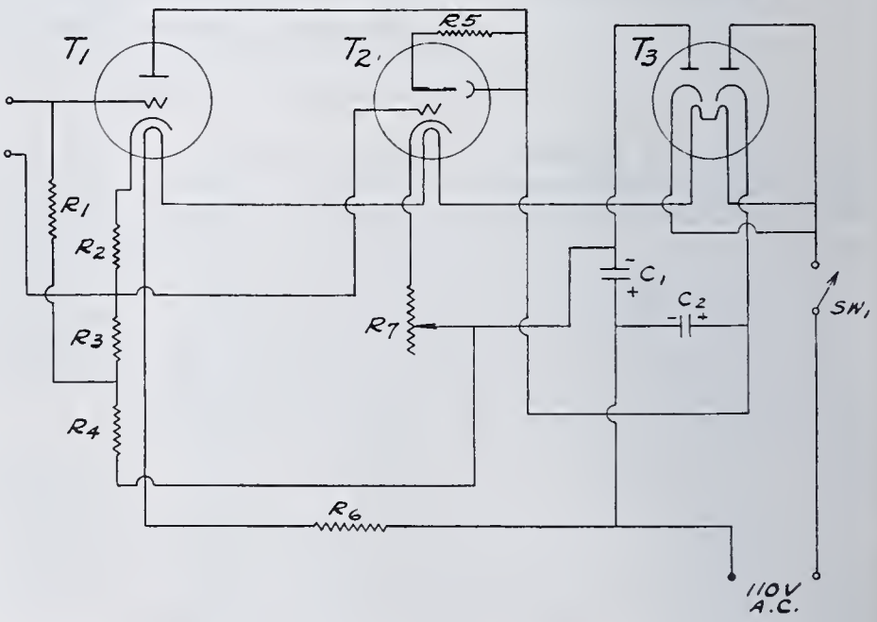

FIGURE 1. WIRING DIAGRAM FOR ELECTROMETRIC APPARATUS

R_1. 100,000-ohm fixed resistor, 0.5 watt
R_2, R_3. 1000-ohm fixed resistors, 1 watt
R_4. 25,000-ohm fixed resistor, 1 watt
R_5. 500,000-ohm fixed resistor, 1 watt
R_6. 350-ohm resistor (in line cord)
R_7. 25,000-ohm volume control
T_1. 6F5
T_2. 6E5
T_3. 6H6
SW_1. Single-pole single-throw toggle switch
C_1, C_2. 4-μf. electrical condenser, 450 volts

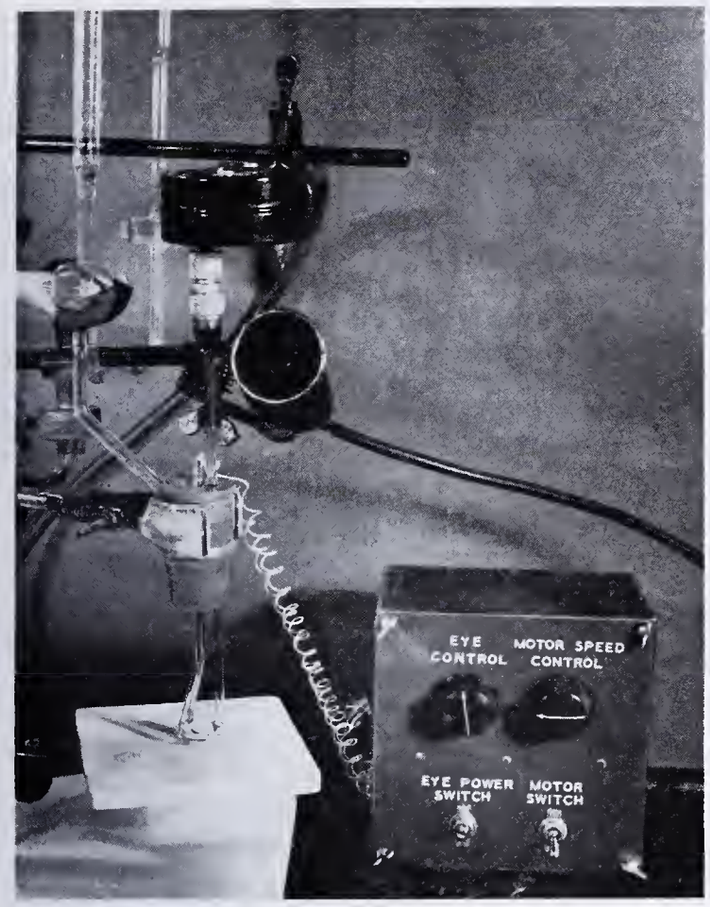

FIGURE 2. ELECTROMETRIC PORTION OF APPARATUS

beyond this point. The standard methanol is added at a rate of 1 to 2 drops per second until the eye starts to close, when the addition of methanol is stopped. The eye will completely close within 2 to 5 seconds. This slight lag is a characteristic of the reaction and has been observed by other workers using both the potentiometric and dead-stop methods.

Since it is essential that atmospheric moisture be carefully excluded from the reagents, an all-glass Schilling buret (Ace Glass Co., Catalog No. 3325) is used to dispense the Karl Fischer reagent. The methanol solution of water used for the back-titration is measured in a buret with a two-way stopcock, using a 2-liter bottle for a reservoir. The tip of this buret was modified by sealing on a tip of the shape necessary to fit the Bakelite stopper used to support the electrodes and prevent the entrance of atmospheric moisture. Both reagent reservoirs and burets are protected from atmospheric moisture with drying tubes filled with Drierite.

The reagents and the Bakelite stopper are the same as those used by Almy, Griffin, and Wilcox (*1*). The Karl Fischer reagent is prepared so that the molar ratio of iodine–sulfur dioxide–pyridine is 1:3:8.

Procedure

Weigh a sample containing 50 to 150 mg. of water into a titration flask. A 150-ml. extraction flask is convenient for this titration. If the sample is a solid or a viscous liquid, add 50 ml. of anhydrous methanol to serve as a solvent. The water content of this methanol must be determined by titrating a 50-ml. portion and correcting the results accordingly.

If the solid is insoluble in methanol, allow the sample to stand in contact with the solvent for 30 minutes. This procedure is adequate for finely divided solids such as wood pulp, nitrocellulose, and cotton linters. For dense granular samples such as proteins and ammonium nitrate, the methanol and sample must be heated to boiling, stoppered, and cooled to room temperature before titrating with the Karl Fischer reagent.

Add the standardized Karl Fischer reagent to the sample until this reagent is present in a 1- to 2-ml. excess, indicated by the change of color of the solution being titrated from pale yellow to dark brown. Place the titrating flask beneath the standard methanol buret, adjust the "magic eye" as described above, and titrate until the eye closes.

In making the calculations, the equivalence factors of the solutions are most conveniently expressed as milligrams of water per milliliters of solution.

TABLE I. ANALYSIS OF KNOWN SOLUTIONS OF WATER IN METHANOL

Sample No.	Water Present	Water Added	Total Water	Water Found	Difference
	Mg.	*Mg.*	*Mg.*	*Mg.*	*Mg.*
1	11.7	56.5	68.2	67.3	−0.9
2	11.7	47.7	59.4	59.5	+0.1
3	11.7	55.0	66.7	66.8	+0.1
4	11.7	139.1	150.8	151.3	+0.5
5	11.7	121.4	133.1	132.3	−0.8
6	11.7	123.2	134.9	134.3	−0.6
7	11.7	168.2	179.9	179.5	−0.4
8	11.7	184.6	196.3	196.4	+0.1

Application of Method

The Karl Fischer method, using this electrometric apparatus for determining the end point, has been applied in this laboratory to the determination of water in a variety of samples, including such liquids as methanol, ethanol,

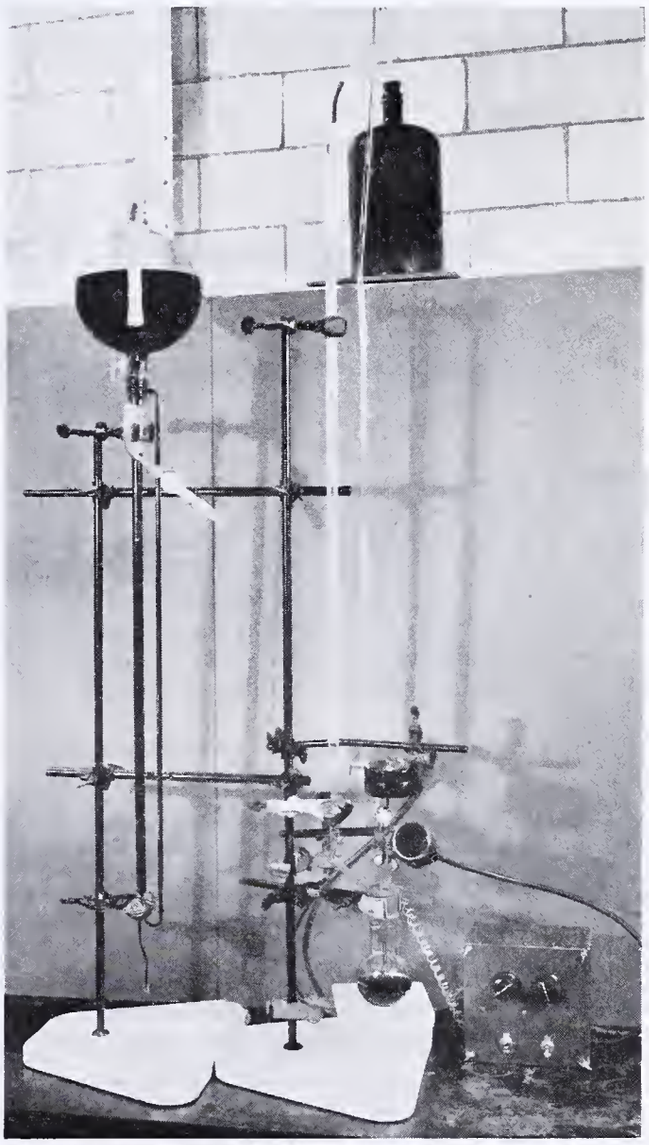

FIGURE 3. COMPLETELY ASSEMBLED ELECTROMETRIC APPARATUS

Table II. Comparison of Electrometric Karl Fischer Method with Other Methods

Sample	Water Found: Karl Fischer	Water Found: CCl_4 Distillation	Water Found: Oven
	%	%	%
Protein	11.1, 11.1	11.1, 10.8	10.9, 10.9
Vinsol resin	1.2, 1.2	....	1.4, 1.4
Pine oil	0.66, 0.64	0.5	.....
Nitroglycerin	0.28, 0.29	0.29[a]	.....
Wood pulp	6.5, 6.6	....	6.3, 6.3
Nitrocellulose	2.8, 2.8	2.9	.....
Glycerol	2.6, 2.6	2.6[b]	.....
Rosin size	30.4, 30.5	30.9	29.3, 29.3
Ammonium nitrate	0.26, 0.26	....	0.33, 0.36

[a] By desiccation method.
[b] By specific gravity.

glycerol, pyridine, nitroglycerin, solutions of resins, pine oil, and pinene, and in solids such as protein material, wood pulp, cotton linters, nitrocellulose, and ammonium nitrate. The results of the determination of water in known mixtures of methanol and water are given in Table I. A comparison of the results obtained on several miscellaneous materials by the Karl Fischer method and by other standard methods is given in Table II.

The possibility that highly unsaturated compounds will interfere with the titration because of the addition of iodine to the ethylenic double bonds has been suggested by earlier investigators. Others observed no interference of this type. The latter conclusion was confirmed by the authors. For example, a 10-ml. excess of the Karl Fischer reagent was added to the sample of pine oil and the mixture allowed to stand for 14 minutes before back-titrating. Under these exaggerated conditions, which are never encountered in a normal analysis, a value of 0.67 per cent water was found, whereas by the regular procedure a value of 0.65 per cent was obtained. A similar experiment with a sample of pinene likewise showed that a negligible absorption of iodine took place.

Summary

An improved apparatus for the electrometric determination of the end point in the Karl Fischer method has been constructed and applied to the determination of water in a variety of materials. The moisture content of unsaturated compounds can be successfully determined by the Karl Fischer method.

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Presented before the Division of Analytical and Micro Chemistry at the 105th Meeting of the American Chemical Society, Detroit, Mich.

An Improved Vapor Thermoregulator

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The improved thermoregulator described in this paper is especially designed for accurate control of the temperature of small insulated rooms. It has advantages over the one previously described by the author (*2*) in that it is easier to make; has a much wider temperature range as well as a greater sensitivity (±0.005° C.); and does not require the use of a metallic bellows, which tends to become brittle after continuous use owing to the action of mercury vapor on the brass.

Design of Regulator

The improved thermoregulator (Figure 1), except for parts of the adjustment mechanism, is made of Pyrex. Essentially, the regulator is a modified U-tube manometer filled with mercury. The levels of the mercury in arms *A* and *B* are controlled by the change of vapor pressure of ethyl ether with the change in temperature. At the upper part of *B* is a small evacuated bulb (about 2 cm. in diameter) containing an electric contact point, *C*, while the upper part of *A* is connected to a thin-walled bulb, *F* (about 4 cm. in diameter and with a wall thickness of about 0.15 mm. at the bottom), containing some liquid ether. For contact point *C*, it was found best to use a fine phonograph needle connected, by means of a steel coupling, to the tungsten wire sealed through at *U*.

The adjustment mechanism connected to the lower part of this modified manometer is similar to that used in the previous instruments described by the writer (*2*, *3*). It consists of a closely fitting Fernico alloy plunger, *L* (about 0.015 mm. smaller than the bore), inside a very uniform-bore (±0.001-mm.) glass tube, *J* (8.04-mm. bore, 50 mm. long). The movement of the plunger is regulated by a finely threaded brass rod through a brass plug fastened to the top of the glass tube by means of wires around the projections on the side of the tube.

K is a mercury reservoir (25 × 35 mm.) with an opening, *Q*, for removing or introducing more mercury if necessary. Below *J* is another reservoir, *M* (10 × 2 cm.), holding enough mercury to take care of any change in levels in *A* and *B* occasioned by a sudden drop in temperature, so no air can be sucked into the upper parts of these tubes.

In order to make it possible for the adjustment mechanism to operate at atmospheric pressure while the upper part of *B* is evacuated and that of *A* is under the vapor pressure of ether, it is necessary to make the length of the mercury column between *C* and the middle of *J* about 800 mm., which is greater than the highest atmospheric pressure ordinarily encountered.

Filling and Adjustment

After assembling the various parts, which should be thoroughly cleaned and dried, into a unit, the thermoregulator is filled with mercury in the following manner: With stopcock *O* turned off and *G* opened to bulb *H*, and the Fernico plunger raised above *J*, the instrument is connected to a good mercury pump at *D* and evacuated thoroughly. Mercury is then distilled into the instrument from flask *E* until reservoir *K* is about three-fourths full. After the instrument has been sealed off at *R*, air is introduced into the space above the mercury in the adjustment mechanism side by carefully opening stopcock *O* until the mercury levels in *A* and *B* rise nearly halfway up in their respective tubes. At the same

time mercury in K is allowed to run into J to keep the mercury level from going down much below J; otherwise air might accidentally get into tubes A and B, which would render the instrument unworkable. To prevent accidentally letting too much air into the apparatus, a cork, P, with a small slot at the side is placed in the inlet tube above O.

Next, about 1 ml. of dried ethyl ether, which has been stored over mercury to remove any peroxide, is transferred through I by the method previously described (*3*) into bulb F, the bottom of which is kept in a cold-water bath. With stopcock G turned off and the water bath replaced by some powdered dry ice to lower the vapor pressure of the ether in the system to about 2 cm. of mercury, the instrument is sealed off at S.

After the dry ice has been removed and the ether in F has come to room temperature, more air is admitted through O until the pressure inside the adjustment mechanism is equal to atmospheric pressure, so cap N can be removed. The manometer reading, shown by the difference between the mercury levels in A and B, is then equal to the vapor pressure of ethyl ether at room temperature. The thermoregulator is now ready for use.

Suppose, for example, it is desired to operate a constant-temperature room at 30° C. As the room is being warmed up to the desired temperature, the vapor pressure of the ether in the system increases and forces some of the mercury back into K, as the Fernico plunger is still above J. When the room temperature reaches a little above 29° C., the Fernico plunger is lowered into J so as to force the mercury level up in B, almost to make contact with C. The amount of mercury in the system can be adjusted so that the Fernico plunger is operating in about the middle of J, when the desired temperature is reached. In case it is necessary to put some mercury back in K, it is best to put N in place again after the plunger has been raised above J and apply suction to O. Mercury is withdrawn from the reservoir by simply tilting the instrument. The final adjustment is accomplished by slowly lowering the plunger until the mercury in A barely makes contact at C when the room temperature is exactly at 30° C. The plunger is then locked in position by means of the lock nut, T. Sufficient time should be allowed for the mercury in the system to come to equilibrium.

Sensitivity and Temperature Range

Since glass is not a good thermal conductor, the sensitivity of the instrument depends largely on the thickness of the wall of bulb F. It should be as thin as possible, but strong enough to stand the pressure encountered at the temperature range at which the instrument is intended to be used. For this reason, this bulb should be tested before being sealed to the instrument.

The lower limit of temperature range of this type of thermoregulator is the freezing point of mercury (−38.87° C.), but at this temperature the sensitivity of the instrument is greatly reduced, since the vapor pressure of ethyl ether at −40° C. changes more slowly with the temperature than at 30° C. For such low temperature, therefore, a liquid such as ethyl chloride is more suitable than ethyl ether. The upper limit of the temperature range is determined by the lengths of A and B. For this particular instrument, the upper limit is a little above 40° C., because the distance between the contact point, C, and the bottom of A is about 940 mm., while the vapor pressure of ethyl ether at this temperature is about 921 mm. If the instrument is intended to be used for higher temperatures, arms A and B should be made longer and the wall of F should be made thicker to stand the correspondingly higher pressure. In place of the glass bulb, F, however, a thin-walled bulb or tube of some metal that does not react with mercury may be connected to the instrument by means of a Fernico coupling. This will permit higher operating temperatures and at the same time give improved sensitivity due to the better thermal conduction of the metal.

This improved thermoregulator together with a sensitive relay (*1*) was able to maintain a constant-temperature room at 30° ±0.005° C. at the regulator as shown by a differential thermometer. In order to do this, the heat input should be properly balanced with the heat loss of the room, so that there is practically no lag in the heating system.

In case it is desired to lower the temperature in the room, the Fernico plunger is raised above tube J and more mercury from the reservoir is added to the system until the plunger is again operating at about the middle of J when the mercury makes contact at C at the desired temperature.

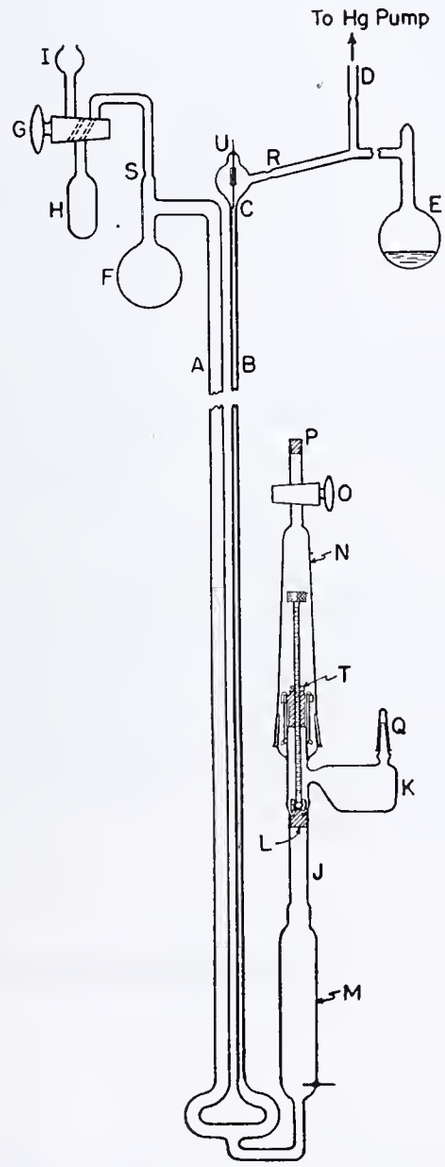

FIGURE 1

In the summer, when the outside temperature was 35° C. or more, it was possible to maintain in a well-insulated (5-cm., 2-inch, cork insulation) room (7 × 13 × 10 feet) to ±0.03° C. at the regulator, with the aid of a small refrigerator unit and two thermoregulators of this type, one controlling the heater and the other the cooling unit. In order to accomplish this, the heat input was carefully balanced against the cooling unit and fans were placed at different levels of the room to ensure proper circulation. Temperature in reaction chambers located on opposite sides of the room showed the same temperature variations.

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Vacuum Distillation Equipment for Volatile Solids

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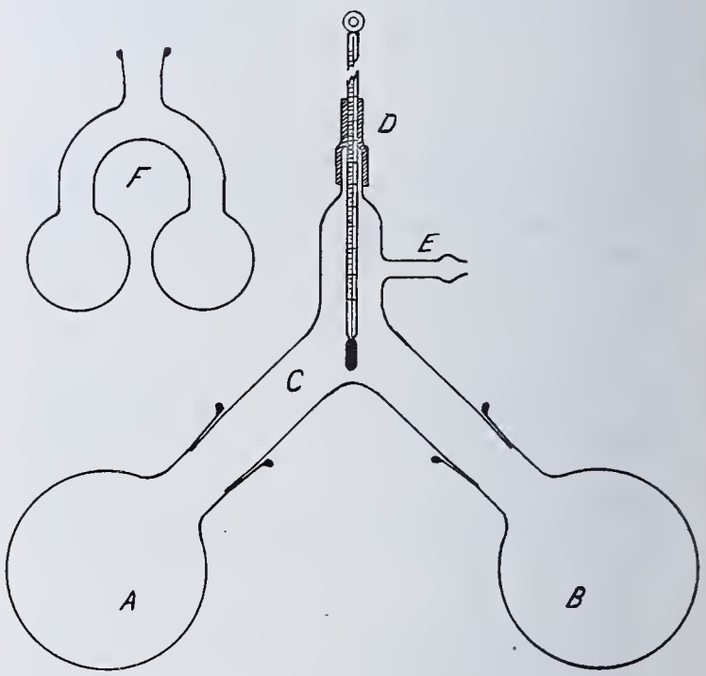
DURING the preparation of a quantity of aluminum ethoxide (*1*) considerable difficulty was encountered in the final purification when the usual vacuum distillation equipment was used. Troublesome condensation, even leading to complete clogging in the delivery tubes, often prevents distillation. Special equipment failed to give satisfactory results (*3*). Furthermore, where the product must be repeatedly distilled there is considerable loss and possible contamination during transfer of the distillate from receiver to original flask. Similar conditions are likewise encountered when distilling benzophenone, phenyl cinnamate, and similar compounds. The apparatus, as illustrated, was designed and constructed to eliminate many of these difficulties.

Two round-bottomed flasks, *A* and *B*, of suitable capacity (50 to 500 ml.) are attached to a glass Y-tube, *C*, of large diameter (15 to 25 mm.). The upper end of this Y-tube is sealed to a short length of 8-mm. glass tubing, so that it may be attached to a thermometer by a small section of rubber tubing at *D*. A convenient side arm, *E*, is provided for evacuating the entire equipment.

The solid to be distilled is placed in flask *A*, the vacuum is applied, and flask *B* is cooled with running water. The application of heat to *A* causes the solid to distill, the temperature being recorded as usual on the thermometer. If the distillation is to be repeated, *A* is now thoroughly cleaned, the vacuum is again applied, *B* is heated, and *A* is now cooled as the receiver. This process may be repeated indefinitely. In case it becomes necessary further to subdivide the distillate at some definite temperature, the flask being used as the receiver may be replaced by one of the type shown at *F*. A simple turn of 180° will throw either flask into position as the receiver. Rubber stoppers, or preferably ground-glass connections (*2*), may be used throughout in constructing the equipment.



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Identification of Rust on Iron and Steel

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IN SOME investigations in this laboratory it was necessary to identify rust on lubricated ferrous alloys. Visual inspection could not be relied upon because of the similarity in appearance between rust and petroleum gum. Ordinary chemical tests were not applicable, since most of them brought about the removal of some of the base metal along with the rust. Removal of the rust by pressing solvent-impregnated filter or gelatin paper onto the specimen proved unsatisfactory; with all the solvents tried, the base metal was preferentially dissolved. While these tests were unsatisfactory on the whole, those obtained with gelatin paper were much better than those in which ordinary filter paper was used. This suggested the use of gelatin paper moistened with water rather than a rust solvent. Subsequently it was found that gelatin paper moistened only with water was capable of removing an amount of iron rust sufficient for testing, without affecting the metal itself.

Procedure

The gelatin paper was prepared by fixing unexposed glossy photographic paper in sodium thiosulfate solution (200 grams in 1 liter of solution), treating in a hypo-eliminating solution (*1*) and drying. The hypo-eliminator (Kodak Formula HE-1) is prepared as follows:

Water	500 ml.
Hydrogen peroxide, 3%	125 ml.
Ammonium hydroxide, 3%	100 ml.
Water to make	1 liter

After fixing, the paper is washed 30 minutes in running water, immersed for 6 minutes in the above solution, washed for 10 minutes in water, and dried. About 6450 sq. cm. (1000 sq. inches) of paper may be processed in 1 liter of solution. Paper processed in this fashion may be stored in stoppered bottles indefinitely.

To carry out a test the gelatin surface of the dry paper is moistened slightly with distilled water and pressed firmly against the specimen. Continuous pressure on the paper is unnecessary, owing to the inherent adhesive property of the moist gelatin. After 15 to 30 seconds the paper is removed, care being taken to avoid stripping the gelatin coating from the paper base. Should it be impossible to remove the paper without this happening, the back should be moistened with distilled water and allowed to

stand for a minute or so, before another attempt at removal is made.

After removal from the specimen, the test paper is immersed in 10 per cent hydrochloric acid containing 0.05 per cent potassium ferrocyanide. Upon development a Prussian blue pattern of the rusted surface is obtained. The development time should be relatively short (10 to 15 seconds) in the case of freshly rusted surfaces if an accurate rust pattern is desired. With aged or worn rust it may be necessary to extend the time of development to one minute or more. The pattern is usually more distinct if the paper is dried with heat.

The Prussian blue developed in the gelatin has little tendency to diffuse and, contrary to filter paper prints, clear, sharp patterns result. Potassium ferrocyanide is preferred to potassium thiocyanate as a developing agent since the latter tends to give a blurred and indistinct image.

Experimental

Tests performed on solvent-cleaned, specially prepared, rusted iron and steel strips using the procedure described gave satisfactory analytical patterns of the rusted surface. Tests made on clean, freshly polished iron strips failed to give a Prussian blue color even upon prolonged development.

The test apparently is specific for iron oxide; attempts to remove sulfide films from copper or lead specimens were unsuccessful. This was attributed to the fine structure and close adherence of the sulfide film in contrast to the rather flaky and loosely held ferric oxide layer.

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An Improved Salt Bridge for Polarographic and Potentiometric Measurements

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AN EXTERNAL reference electrode is frequently used in potentiometric and polarographic measurements. The connection to the solution is by some type of a salt bridge. The ideal salt bridge, in addition to eliminating the junction potential, should have a low resistance (especially in polarography), should not contaminate the solution being used, and should be easy to handle. A number of bridges have been described in the literature, none of which incorporates all the desired features. The inverted-U bridge of Irving and Smith (*2*), with ground-glass plugs at the ends, has been widely used in potentiometric work but has an undesirably high resistance for polarography if the reference electrode is to be used as the anode. A bridge of this type, 40 cm. long, when filled with saturated potassium chloride, was found to have a resistance of about 7000 ohms, some 6500 ohms being attributable to the plugs. Laitinen (*3*) has described an inverted-U bridge with sintered-glass ends which has a lower resistance but which, in common with other all-glass designs (*1*, *4*), is rather cumbersome to use.

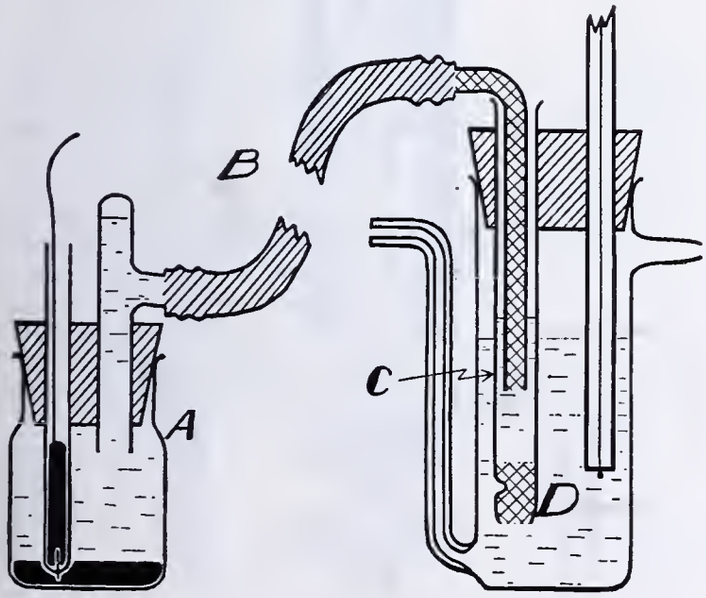

FIGURE 1

The authors have found the very simple bridge illustrated in Figure 1 to be highly satisfactory. The saturated calomel electrode, *A*, is connected to the cell with a piece of ordinary soft rubber tubing, *B*, 6 mm. in inside diameter, filled with saturated potassium chloride. The rubber tube terminates in a short length of glass tubing filled with 3 per cent agar jell, also saturated with potassium chloride. When it is to be used, the free end of the bridge is simply plugged into the cell. The reference electrode may be kept permanently out of the way and the necessity of simultaneously adjusting two cells in a thermostat to connect with an unbendable glass bridge is eliminated.

Figure 1 shows how an intermediate agar plug, *D*, having the same composition as the cell liquid, may be used if contamination with potassium chloride is undesirable. If traces of potassium chloride are not objectionable, the free end of the bridge may be inserted directly into the solution. For this purpose it is convenient to have a thin, coarsely-sintered glass plug at the tip of *C*. The agar is still desirable to prevent mixing by convection. The end of the bridge is kept in potassium chloride solution when not in use.

The resistance of such a bridge 56 cm. long was found to be only 600 ohms. For a polarogram showing a diffusion current of 10 microamperes, the error in half-wave potential due to *IR* drop in the bridge would be about 3 millivolts. This error is negligible for most purposes and may, of course, be decreased by the use of a shorter bridge or larger tubing. If exact values of half-wave potentials are desired, the resistance of the cell and bridge may be measured and the correction applied whenever necessary. For potentiometric titrations, in which a low bridge resistance is not essential, plug *D* is conveniently replaced by one of ground glass, as in the bridge of Irving and Smith (*2*).

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A Briquetting Press and Electrode Loader for Spectrochemical Analysis

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An inexpensive and easily constructed apparatus is described, for briquetting a powdered sample for spectrographic analysis and loading the briquet into an electrode crater without subsequent manual handling. The apparatus minimizes contamination and loss of sample, as well as danger of electrode breakage upon loading.

SAMPLES submitted for spectrochemical analysis are often in the form of powder or material which must be brought to a powder in preparation for the analysis. This is particularly true in the case of rocks, ores, slags, refractories, ashes, residues, ceramic products, and pigments. The dried residue of a dissolved metal or alloy is also sometimes used for analysis.

When a powdered sample is placed in the crater of a carbon or graphite electrode, some difficulties may be encountered. An unpacked sample requires a deeper electrode crater than a tightly packed sample, and an excessive amount of electrode wall must be consumed if the sample is burned to completion. A powdered sample placed loosely in the crater does not burn so uniformly as a sample packed in the electrode under pressure. A light and fluffy powder, or one containing certain compounds, tends to be blown from the crater during arcing. Considerable time is often spent in transferring a weighed sample to the crater without any loss of sample. Preburned electrodes tend to be fragile and extra care must be taken in filling them.

Packing the powdered sample into the electrode crater or briquetting the sample into a firm pellet and placing the pellet in the crater has become common practice. The packing operation is performed by means of a steel rod (*5*); and the briquetting is done with the aid of a punch and die (*2*, *4*), a micro pellet press, or a briquetting press recently described (*1*). In all these briquetting methods the pellet must be handled in placing it in the electrode crater.

The apparatus described here briquets the sample, discharges the pellet directly into the electrode crater, and presses the pellet firmly into the crater without subjecting the pellet to handling by the operator. Repeated checks have shown a negligible loss of sample during these operations. The apparatus is inexpensive, requires very little metal for its construction, and can be easily built in any well-equipped machine shop. The sample to be briquetted may be a powdered sample or a mixture of sample and other material added to serve as arc stabilizer, binder, or carrier of the internal standard. Electrodes prepared by means of either of the two most commonly used electrode cutting tools (*3*, *5*) may be used.

Apparatus

DESIGN DETAILS. Figure 1 shows the construction and Figure 2 shows the apparatus assembled and unassembled.

The base, *A*, is a circular piece of 0.25-inch steel plate, 2.75 inches in diameter, supported by three 0.25-inch steel legs, *B*, 1 inch in length from the bottom of the base. The electrode holder, *C*, is a hollow bolt with a 0.75-inch hexagonal head, *D*, 1.375 inches long and having a 0.5-inch threaded outside diameter. It has a 0.25-inch inside diameter which is threaded through the head end to a depth of 0.5 inch. The upper end is beveled flat to fit tightly against the briquetting mold. The bolt is screwed up tight through the center of the base. The electrode height adjuster, *E*, is a threaded steel rod 0.75 inch in length and 0.25 inch in diameter, at one end of which is a knurled nut, *F*, 1 inch in diameter and 0.25 inch thick. The adjuster screws into the electrode holder from below.

The briquetting mold, *G*, is a cylindrical piece of 0.875-inch steel rod 2.375 inches in length. A threaded hole 0.5 inch in diameter and 0.688 inch in depth is bored in the center of the lower end of the mold. Two rectangular slits, *H*, 0.25 inch wide and 0.688 inch in length, are cut on opposite sides of the threaded end of the mold. A 0.188-inch hole is bored in the center of the upper end of the mold and extends through the mold to the threaded end. The top of the mold is reamed out to a funnel shape 0.25 inch deep and 0.75 inch in diameter. A 1.5 × 0.25 × 0.125 inch steel plate, *I*, with rounded ends fits into the slit at the bottom of the mold and separates the top of the electrode holder from the bottom of the 0.188-inch hole in the mold when the mold is screwed down firmly over the electrode holder. A tool steel rod, *J*, 0.188 inch in diameter and 2 inches in length, slightly concave at the lower end, and having a head, *K*, at the upper end made of a circular piece of 0.25-inch steel 1.44 inches in diameter, serves as a plunger. A small wire spring, *L*, 1.75 inches in length, compressible to 0.688 inch, is placed over the plunger and is held in place by the plunger head.

OPERATION. The briquetting mold is removed from the electrode holder and the plunger is removed from the mold. A 0.25-inch spectroscopic electrode, less than 1.25 inches in length, having square-cut ends and a crater of desired depth, is placed in the electrode holder with the crater end up, but not extending above the top of the electrode holder. The mold is screwed down over the electrode holder, the steel plate is placed in its slot, and the mold is tightened. The electrode is

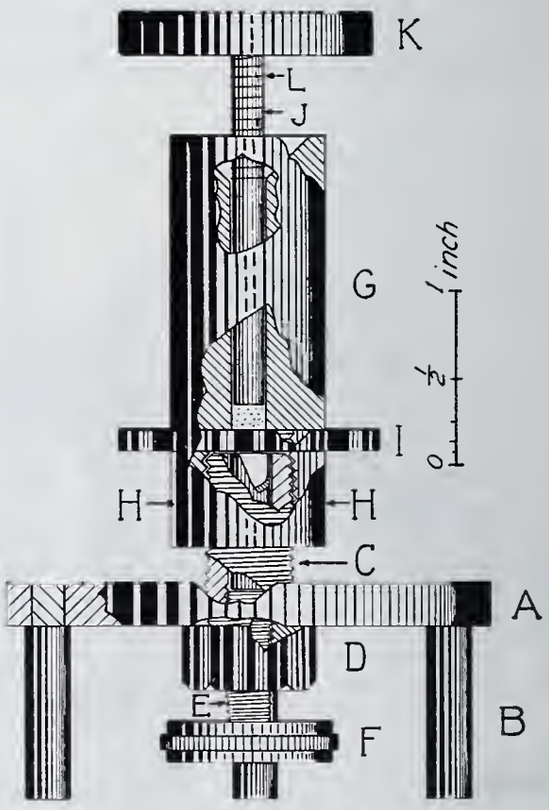

FIGURE 1. CONSTRUCTION OF APPARATUS

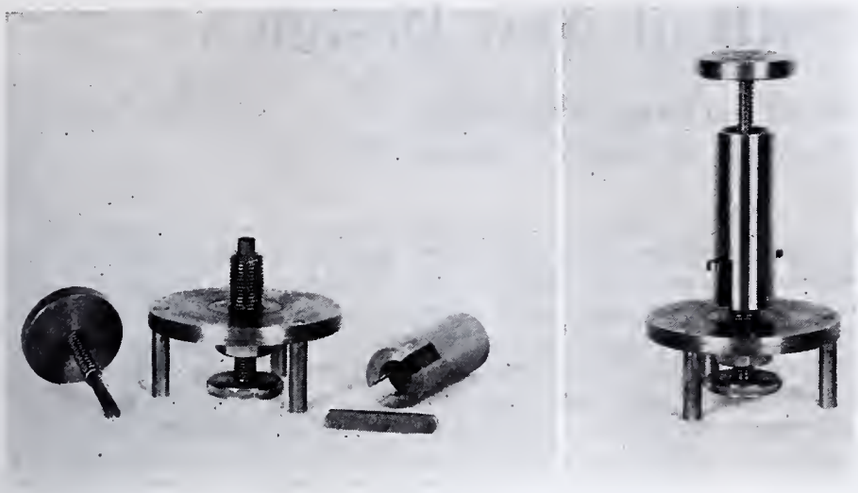

FIGURE 2. BRIQUETTING PRESS, UNASSEMBLED AND ASSEMBLED

raised by turning the height adjuster until the top of the electrode presses firmly against the bottom of the steel plate. The powdered sample or sample mixture is placed in the 0.188-inch hole in the top of the mold, using the top of the mold as a funnel, and is brushed into the mold with a camel's-hair brush. If it is desirable that the sample not touch the upper part of the sidewalls of the mold, a small glass funnel may be used to introduce the sample into the bottom of the mold. The plunger is placed in the mold and pressure is applied to the plunger head, molding the sample between the bottom of the plunger and the top of the steel plate, *I*.

The spring raises the plunger from the sample when the pressure is released. The mold is loosened one turn, the steel plate is removed, and the mold is screwed down until the inside of the mold is in firm contact with the electrode holder and the top of the electrode. A slight pressure on the plunger head discharges the pellet into the electrode crater and additional pressure forces the pellet firmly into the crater. The mold is removed from the electrode holder and the height adjuster is turned up until the filled electrode projects sufficiently from the top of the electrode holder to be easily removed.

The apparatus as described is fitted to electrodes of 0.25-inch diameter with drilled craters larger than 0.18 inch, but dimensions can be adjusted for construction of a similar apparatus fitted to electrodes of other size. The apparatus is easy to take apart and can be cleaned with the aid of a small brush and pipe cleaner. Fragile, preburned electrodes may easily be filled without breaking. The samples may be briquetted by hand pressure or by means of a press operated under known pressures (*4*).

Acknowledgment

The authors wish to thank Wallace Lowry of the Oregon State Department of Geology and Mineral Industries for technical assistance.

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A Glass Laboratory Pump for Gases or Liquids

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AN ALL-GLASS pump, which is leakproof and simple in construction, has been found satisfactory for circulating gases or liquids in an experimental apparatus. The pump is a reciprocating piston with two check valves arranged as indicated in Figure 1. Features of construction are the mercury-sealed 5-ml. Pyrex hypodermic syringe that serves as a piston, the vacuum-operated reciprocating motor of the automobile windshield-wiper type, and the mercury-loaded glass check valves. The check valves, which are bulbs 10 mm. in diameter on a 15-mm. length of 4-mm. tubing, are ground to form a gastight seat on 8-mm. tubing. A small amount of mercury sealed into each check valve adds weight and ensures rapid and secure seating of the valve. Mercury from the seal around the plunger does not penetrate the piston.

The pump delivers gas at 230 ml. per minute against a head of 1 cm. of mercury; the limiting head for gas is 25 cm. of mercury. The piston with its mercury seal is gastight. Liquids are delivered at heads limited only by the power of the motor.

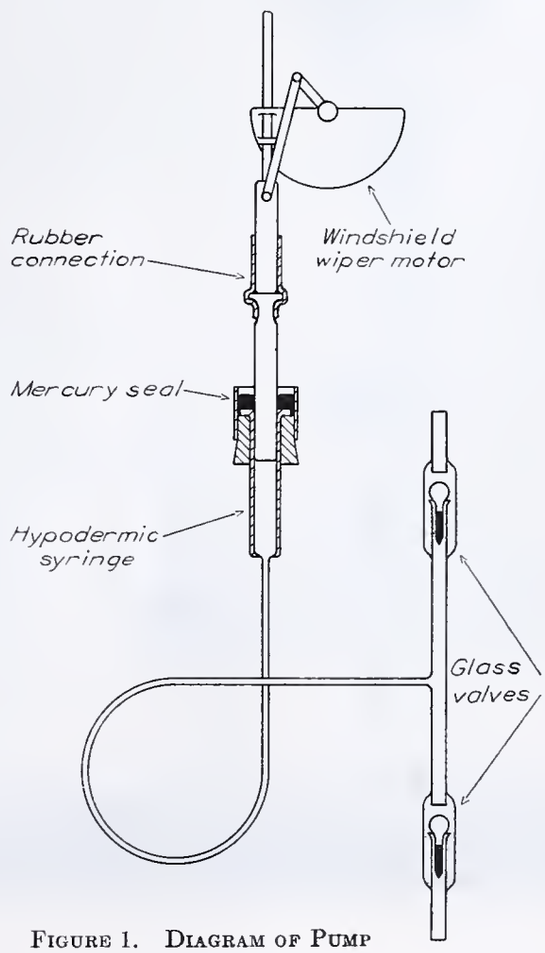

FIGURE 1. DIAGRAM OF PUMP

A Molecular Still of New Design

F. W. QUACKENBUSH AND HARRY STEENBOCK
College of Agriculture, University of Wisconsin, Madison, Wis.

A cyclic still of simple design for use in the purification of either large or small quantities of material is described. The evaporator surrounds the condenser, thus providing a large surface area for evaporation and a relatively small area from which the condensate must drain. During a distillation a magnetically driven rotor spreads the incoming distilland in a thin film over the evaporator surface. The condenser is attached through a ground joint and its easy removal makes possible a rapid quantitative collection of either liquid or solid distillates. The still is glass-surfaced throughout and is therefore well adapted for use in autoxidation studies. This type of still, besides contributing to greater efficiency in distillation, makes the technique of molecular distillation applicable to a wider variety of laboratory problems than others hitherto in use.

IN THE application of molecular distillation to the separation or purification of compounds, two recognized technical problems involve (1) the continuous renewal of the surface of the distilland, and (2) the removal of the distillate from the condenser. The first is of importance because the process of molecular distillation is essentially evaporation from the surface of the distilland and it is evident that only those molecules which reach the surface have the opportunity to pass to the condenser during the heating interval. It is therefore essential that the surface be continuously renewed to prevent the less volatile components of the distilland from imprisoning molecules which would normally distill if allowed to reach the surface. Such imprisonment is exemplified in the pot type of still, in which a thick layer of distilland is commonly employed without special provision for effecting surface renewal. In such a still relatively high temperatures are required to effect distillation and since the heating is necessarily continuous, heat-labile compounds are subjected to a great risk of decomposition. Nevertheless, the degree of fractionation is low.

A considerable advance in distillation technique has resulted with the shifting of the evaporator from the horizontal to a vertical position, in which form the distilland is allowed to flow over a cylindrical or dome-shaped evaporator (*3–6*). Such vertical-surface stills depend upon gravitational flow to control the thickness of the layer of the distilland and to effect renewal of the distilling surface.

One of the chief problems is to induce the distilland to spread over the evaporator. In attempting to solve this problem, experimenters have resorted to the use of concentric wire gauze distributors and spiral embossing of the evaporator surface (*3*), spirally wound wire (*5*), or fragment glass which is fused to a glass evaporator to roughen its surface (*1*). While these devices are reported to effect spreading, it is to be expected that they should obstruct the free flow of the distilland and thus create zones of variable thickness and regions of sluggish flow with consequent subjection of localized areas to a relatively long period of heating. A further difficulty which may arise in simple gravitational flow is that of stratification of the distilland (*2*), the inner strata nearest the evaporator moving very slowly while the colder outer strata, from which evaporation is possible, move with relative rapidity. This difficulty is likely to become of importance with a distilland of high viscosity and especially at the lower temperatures.

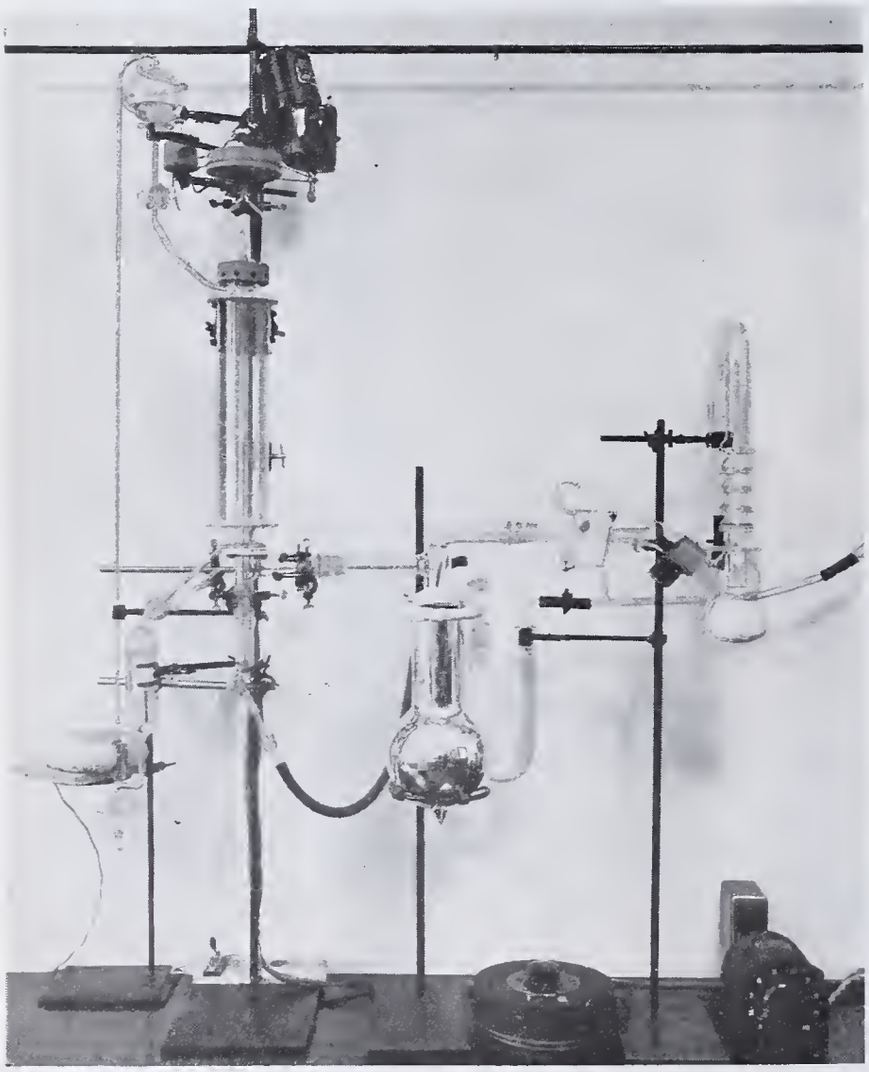

FIGURE 1

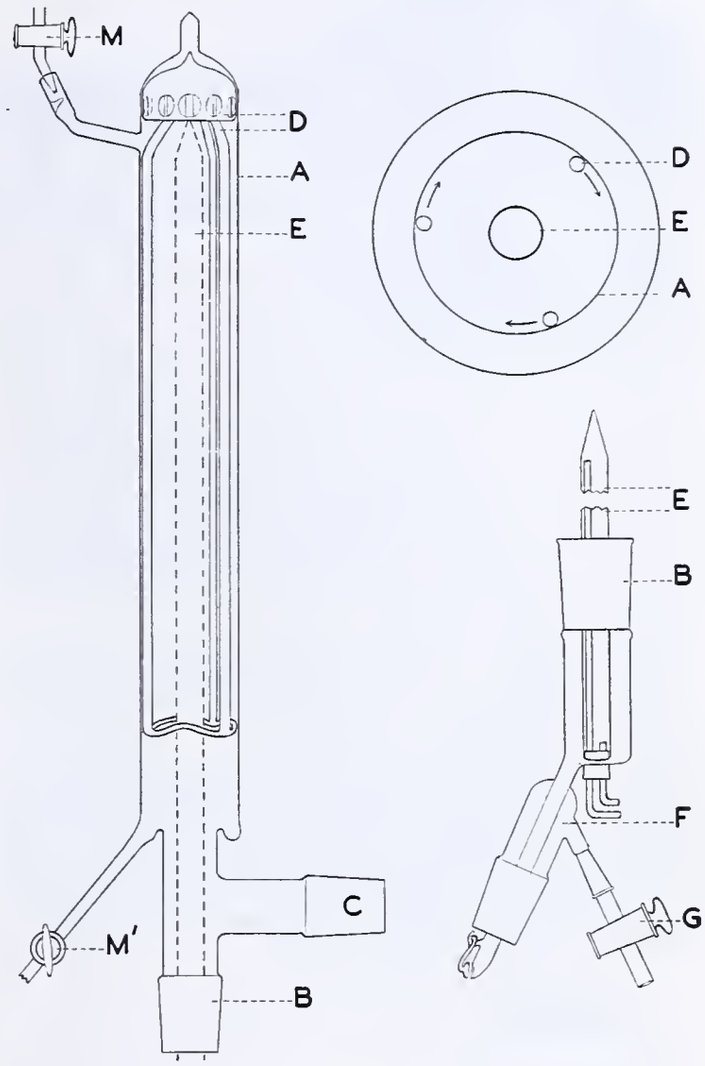

FIGURE 2. DISTILLING CHAMBER

A, evaporator; *B*, *C*, joints for attaching condenser and cold trap, respectively; *D*, rotor; *E*, condenser; *F*, fraction cutter; *G*, cock connecting to vacuum line; *M*, *M′*, cocks connecting to reservoirs.

The second problem, that of removal of the distillate from the condenser, is one of particular importance in laboratory-scale operations. In the vertical surface stills which have been employed, the distillate is spread over a large surface, since the condenser commonly surrounds the evaporator and must therefore have a larger surface area than that of the evaporator. In laboratory operations the total distillate may consist of as little as 1 gram or even 1 mg. of material. Furthermore, for some purposes it may be desirable to divide the distillate into a number of fractions. If it happens to be a liquid, some of it may find its way through the delivery tube and fraction cutting device into the receiver. However, if it is a solid or semisolid it is likely to remain quantitatively on the walls of the condenser. To circumvent this difficulty Hickman (*3*) introduced the use of a constant yield oil, the function of which was to entrain the distillate and carry it out of the still. In effect, this technique involves the transfer of the distillate from one oil to another, from which it is usually not more readily removable than from the first.

A third problem in still construction, the elimination of exposed metallic surfaces, arose in this laboratory in the application of molecular distillation to the study of autoxidation of fats. Since it is well known that mere traces of certain metals markedly accelerate autoxidation, it seemed inadvisable to attempt such studies with a still in which the fats were continuously in contact with metallic surfaces.

Effective advances have been made toward the solution of these three problems in a still now in use in this laboratory. A description of the construction and operation of the still (Figure 1) are given here.

Construction of the Still

The functional part of the still, the distilling chamber, consists of (1) a cylindrical evaporator which is electrically heated from the outside, (2) a magnetically driven rotor which spreads the distilland over the inner surface of the evaporator, (3) a removable condenser which serves as a bearing for the rotor, and (4) a fraction cutter for the removal of liquid distillates. The chamber is connected on one side through a dry ice trap to an oil diffusion pump outfit (Cenco No. 93,270A) and a modified McLeod gage, and on the other side to two reservoirs and a magnetic pump.

The details of the distilling chamber are shown diagrammatically in Figure 2. The evaporator, *A*, is a Pyrex tube, 50 × 450 mm., which is closed at the upper end and connects through an annulus to the two male joints, *B* and *C* (₮ 29/42), at the lower end. A side arm from the annulus connects with the lower reservoir, and another side arm near the top of the evaporator connects through a drop-regulator to the upper reservoir. The evaporator is heated by a jacket which contains a series of ten vertical coils comprising a total of 60 feet of No. 22 Nichrome wire. A thermometer is mounted in contact with the evaporator wall and the temperature is controlled by a rheostat in a 110-volt circuit.

The rotor, *D*, consists of three 5-mm. rods fused to a magnet chamber fitted closely to the evaporator surface, with just enough clearance to allow rotation (see cross-sectional view at upper right of Figure 2). The magnet chamber contains a ring of ten permanent magnets (Alnico, General Electric Co., 0.938 cm., 0.375 inch, in diameter and 0.469 cm., 0.188 inch thick) which are held in position by decagonal sheets of asbestos and sealed in the chamber under high vacuum. A conical indentation in the center of the lower surface, ground to fit the tip of the condenser, serves as the bearing for the rotor. The rotor is turned by a rotating magnetic collar which is driven by a variable-speed motor. The collar fits snugly around the outer surface of the evaporator and contains 10 Alnico magnets identical with and placed in juxtaposition to those of the magnet chamber.

The condenser, *E*, is a 14 × 600 mm. tube, the lower end of which is fused to the female part of joint *B*. The condenser and rotor are in position for operation when male and female joints *B* are brought together. The condenser is equipped with inlet and outlet tubes for either air or liquid cooling. The fraction cutter, *F*, has a delivery tube with a check valve extending through the ground joint (₮ 24/40) into the receiving flask (not shown). The check valve is a glass bead ground to fit the opening of the delivery tube. A glass rod attached to the bead forms a hook and eye hinge with a rod fused to the delivery tube directly above, so that the bead rests lightly against the opening. The receiver is evacuated through a stopcock, *G*. With the distilling chamber evacuated, readmittance of air through *G* causes the bead to press firmly into position. An adhering drop of distillate serves as lubricant and seal for the check valve. When the distillate is to be small in quantity or solid in nature, the bead is removed from the hook, and cock *G* is replaced by a glass stopper.

The lower reservoir has a capacity of 160 cc.; the upper, 500 cc. Between the reservoirs is the magnetic pump which is shown diagrammatically in Figure 3. The pump has a glass plunger, *H*, which is ground to fit its cylinder, and two check valves, *I* and *I′*, which are glass balls at the ends of small rods. Within the plunger, sealed under vacuum, is a steel cylinder, *L*, which weighs 14 grams, to make possible its magnetic operation. A stopcock, *J*, serves for the introduction of the distilland into and its removal from the still. For the introduction of the distilland a capillary extension is attached through the ground joint (₮ 10/30). During the distillation this is replaced by a cap. In removing the distilland, the pump is drained by inserting the glass rod, *K*, through the cock, *J*, thus dislodging valve *I* and pushing the plunger upward finally to dislodge valve *I′*.

The pump is operated by an electromagnet which consists of a core of soft iron 20 × 20 × 400 mm. and several hundred turns of No. 16 magnet wire. The current is furnished by a 6-volt storage battery. A circuit breaker to provide intermittent cur-

rent is driven by the same variable-speed motor which drives the rotor.

The stopcocks and ground joints ("No lub" joints, manufactured by the Scientific Glass Apparatus Co., Bloomfield, N. J.) are sealed with a viscous stopcock grease. Leakage is negligible, since the gage shows a pressure of only 10 to 20 microns 24 hours after turning off the pumps.

Operation of the Still

To carry out a distillation the cold trap is immersed in a dry ice–acetone mixture, the mechanical pump started, and the system evacuated to a pressure of 25 microns. The oil to be distilled is then drawn through the capillary into the lower reservoir, and is partly degassed during the entry. With cock *M* closed, the pump is halted momentarily and sufficient air allowed into the system to force the oil into the upper reservoir. The vacuum is re-established, the rotor and magnetic pump are set in motion, and *M* is opened. Degassing is usually complete within 1 to 4 hours, depending upon the nature and quantity of the oil charge. It is hastened if the distilling chamber is warmed. When degassed, the oil is held in the upper reservoir, the oil diffusion pump brought into action, and the distilling chamber heated to the desired temperature. When suitable temperature and pressure are established *M* is opened sufficiently to give the desired rate of flow (usually 100 drops per minute) and the distillation allowed to proceed.

When the distillate is a liquid, and of considerable volume as in the distillation of fatty acids or glycerides, fractions are removed readily by the use of the fraction cutter. However, more often the distillate is a solid or semisolid or the amount of material is so small that it will not flow into the receiving flask. For the removal of a fraction in such cases, cocks *M* and *M'* are closed so as to keep the oil degassed, the pumps are turned off, and nitrogen is allowed to enter the system from a balloon. Joints *B* are disengaged, the condenser is removed, and the distillate is washed quantitatively from the condenser with a suitable solvent such as acetone or benzene. About 10 minutes are required to remove a fraction in this manner and re-establish conditions for continuing the distillation.

Application of the Method

The effectiveness of the still in concentrating a substance from its dilute solution was shown in the analytical distillation of two standard dyes (purchased from Distillation Products, Inc., Rochester, N. Y.), celanthrene red and dibutyl aminoanthraquinone, after the method of Hickman (*3*). The diluent was a residue oil, corn oil from which the volatile constituents had been removed by distillation at 210° C.

In each case 1 mg. of dye was dissolved in 50 grams of the residue oil and distillation was allowed to proceed for 20 minutes, during which time the oil made one complete cycle through the still. The distillate was removed from the condenser with acetone and the quantity of dye determined colorimetrically. Subsequent fractions were removed at progressively increasing temperatures until the dye had distilled completely from the oil.

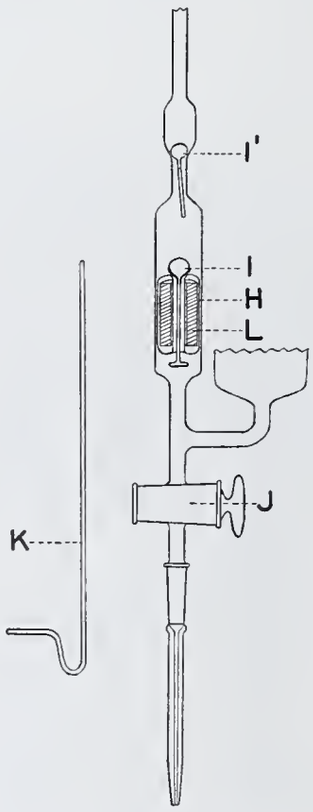

Figure 3. All-Glass Surfaced Magnetic Pump

H, plunger; *I*, *I'*, check valves; *J*, cock for introduction of distilland; *K*, tool for releasing valves to drain pump; *L*, iron cylinder

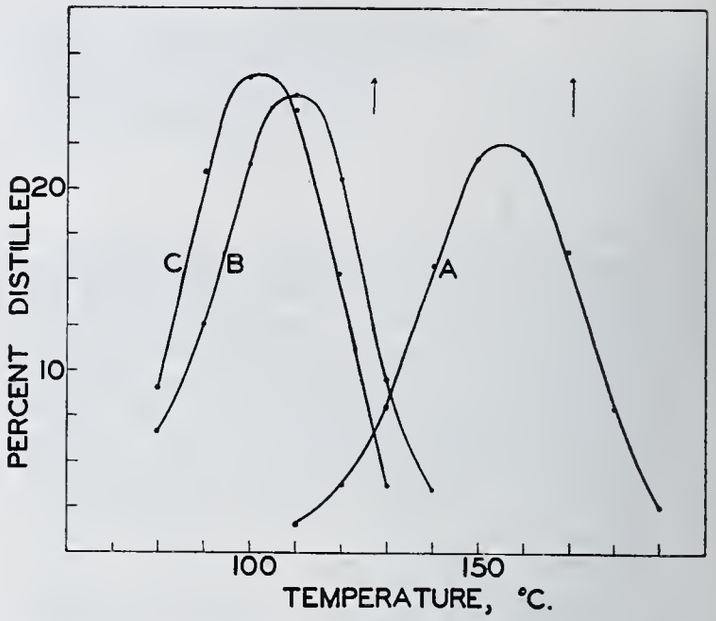

Figure 4. Distillation Curves Obtained with Standard Dyes

A, dibutyl aminoanthraquinone, 20-minute cycle; *B*, celanthrene red, 20-minute cycle; *C*, celanthrene red, 30-minute cycle. Arrows indicate temperature of maxima reported by Hickman for the same dyes with a 10-minute cycle.

The curves thus obtained (Figure 4) showed maxima at 110° and 155° C., respectively, for the two dyes, or 17° and 16° lower, respectively, than those reported by Hickman (*3*), who used a 10-minute cycle. The actual differences in distilland temperatures were probably even greater, since the authors' values represent temperatures within the heating jacket. Under these conditions variable results were obtained with a 10-minute cycle, probably because of the low heat conductivity of the glass evaporator. It is evident, however, that the authors' lower distillation maxima are attributable in part to the longer cycle and in part to the improved spreading of the distilland over the evaporator surface.

In the application of the still to practical problems of the laboratory its adaptability has been further demonstrated. It has been used successfully in the concentration of antioxidants (*7*), tocopherols (*8*), and other vitamins and sterols from natural oils, in analytical distillations, and in the distillation of higher fatty acids, natural fats and oils, and synthetic glycerides.

Acknowledgment

This contribution was made possible by a grant from the Lever Bros. Company and from The Wisconsin Alumni Research Foundation.

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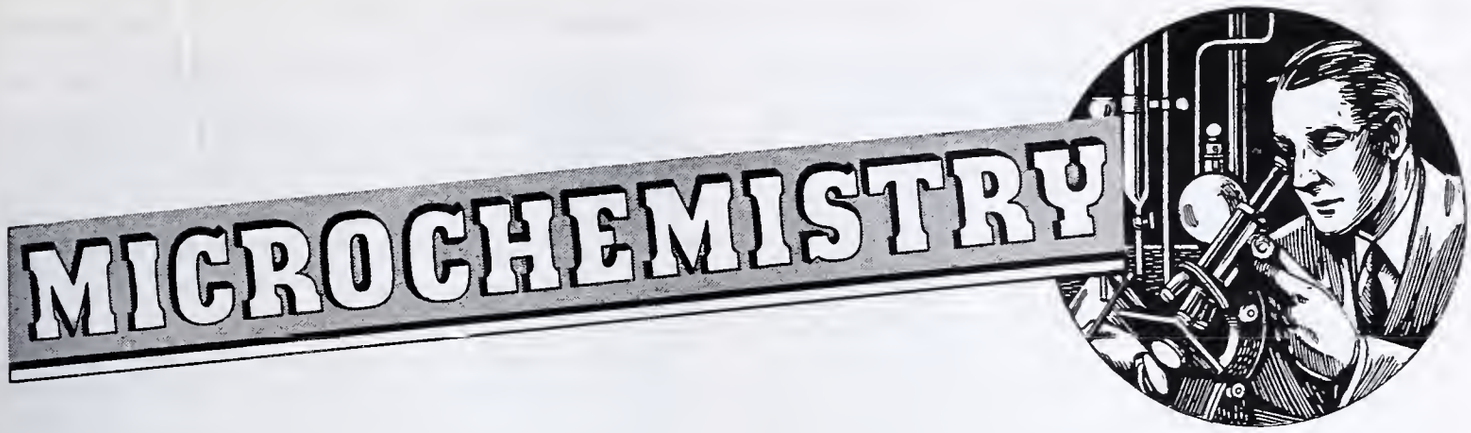

Determination of Nicotinic Acid

Modifications in the Microbiological Method

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THE Snell-Wright microbiological assay for nicotinic acid (*13*) has provided a valuable method for the determination of the vitamin, which has been widely employed in nutritional research. The method has been in use in this laboratory for over 2 years, and has been applied to many hundreds of samples. While the results on the whole have been fairly satisfactory, a number of difficulties have been encountered. This paper sets forth certain modifications in the procedure which have led to more consistent and uniform results, and describes the method in detail as it is now being carried out in this laboratory.

The difficulties that have arisen in the use of the original Snell-Wright method have been associated in the main with the standard curve. When the assay is employed routinely over an extended period of time, it has been the authors' experience that the different standard curves vary considerably, the growth of the bacteria sometimes being so poor that it is scarcely possible to arrive at any results at all. Even the better curves, such as curve 1, Figure 1, are decidedly rounded, and frequently are not linear to levels of nicotinic acid higher than 0.2 microgram. While results read from the higher parts of such curves may be reliable, the nonlinearity of the response raises the suspicion that substances other than nicotinic acid are limiting the growth at the higher levels, and hence implies the possibility of a lack of specificity of the assay.

A minor trouble, which has caused much unnecessary labor in many laboratories, has been the difficulty in securing satisfactorily low blank values. High blanks have been caused chiefly by the presence of nicotinic acid in the casein and biotin concentrates formerly used, and may now be easily avoided.

The discovery that fatty acids interfere with the microbiological determinations of riboflavin (*15*) and pantothenic acid (*10*) naturally raised a question regarding the possibility of similar interference in the determination of nicotinic acid. Fortunately *Lactobacillus arabinosus*, when grown on the nicotinic acid basal medium, seems to be insensitive to fatty acids.

Experimental

COMPOSITION OF THE BASAL MEDIUM. Increasing the glucose and sodium acetate in the Snell-Wright basal medium to 2 per cent of each resulted in a definite improvement in the bacterial response to nicotinic acid (Figure 1). Two per cent glucose alone, however, had no effect (curve 1, Figure 2). Kline (*7*) has reported that increasing the cystine content of the basal from 0.01 to 0.02 per cent brings about a straight-line response over a wider range, and results in higher acid production. In the authors' hands the higher amount of cystine has also proved slightly beneficial (Figure 2).

Isbell (*5*) reported that *p*-aminobenzoic acid must be added to the medium, when the casein hydrolyzates have been heavily treated with charcoal, in order to obtain satisfactory growth. The authors have not been able to observe such an effect, no doubt because of a contamination of some of their reagents with this factor, but have considered it advisable to include *p*-aminobenzoic acid in the modified basal medium.

The composition of the medium finally adopted is shown in Table I. Aside from the exceptions noted above and the use of 0.2 instead of 0.4 part per billion of biotin, the composition is the same as that recommended by Snell and Wright. This modified medium is referred to in the remainder of this paper as the basal medium.

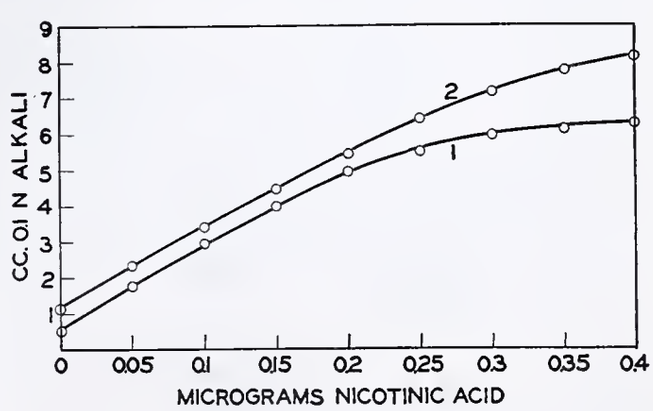

FIGURE 1. RESPONSE TO PURE NICOTINIC ACID (72 HOURS' INCUBATION)

1. Snell-Wright medium
2. Snell-Wright medium modified to contain 2 per cent glucose and 2 per cent sodium acetate

Table I. Basal Medium for Nicotinic Acid Assay

Acid-hydrolyzed casein	0.5%
l(-)Tryptophan	0.01%
l(-)Cystine	0.02%
Glucose, anhydrous	2.0%
Sodium acetate, anhydrous	2.0%
Calcium pantothenate	0.1 p. p. m.
Thiamine chloride	0.1 p. p. m.
Pyridoxine	0.1 p. p. m.
Riboflavin	0.2 p. p. m.
Biotin	0.2 p. p. m.
p-Aminobenzoic acid	0.1 p. p. m.
Adenine	10 p. p. m.
Guanine	10 p. p. m.
Uracil	10 p. p. m.
Inorganic salts solution A	See text
Inorganic salts solution B	See text

Preparation of Stock Solutions. The stock solutions described below are stored in the dark in the presence of chloroform and toluene, and preferably in the refrigerator. The toluene is added until a thin layer is visible over the surface of the solution, and the quantity of chloroform used is such that a few drops are visible at the bottom of the flask.

Acid-Hydrolyzed Casein. One hundred grams of "vitamin-free" casein—e. g., Labco—are twice extracted by stirring 15 minutes at room temperature with 2 to 3 volumes of 95 per cent ethanol and filtering. The casein is then gently refluxed over a low flame with 300 cc. of concentrated hydrochloric acid for 16 to 20 hours. The casein hydrolyzate is concentrated to a paste in vacuo from a 70° to 80° C. water bath, 200 cc. of water are added, and the concentration is repeated. The residue is dissolved in about 700 cc. of water, adjusted to pH 4.0 (yellow-green to bromocresol green, outside indicator) with saturated sodium hydroxide solution, 20 grams of an activated charcoal (Norite A or Darco G-60) are added, and the mixture is stirred for 1 hour at room temperature. The charcoal is removed by gravity filtration, the pH adjusted to 6.6 to 6.8 (bromothymol blue, outside indicator), and the filtrate diluted with distilled water to 1000 cc. Each cubic centimeter therefore is equivalent to 100 mg. of the original casein. The final solution should be no darker than a pale straw color. Any sediment occurring in the casein hydrolyzate on standing may in general be eliminated by autoclaving for 1 hour at 10,500 kg. per square meter (15 pounds per square inch) pressure.

l-Cystine Solution. Four grams of *l*-cystine are suspended in 500 cc. of hot water and the smallest amount of concentrated hydrochloric acid needed to effect solution is added dropwise with stirring. The solution is then diluted to 1000 cc., so that each cubic centimeter contains 4 mg. of *l*-cystine.

l(-)Tryptophan. Two grams of *l*(-)tryptophan are dissolved in about 700 cc. of warm water, with the aid of a few drops of concentrated hydrochloric acid. The solution is diluted to 1000 cc. so that each cubic centimeter contains 2 mg. of *l*(-)tryptophan.

Anhydrous c. p. glucose and sodium acetate are weighed out as needed.

Biotin. The contents of an ampoule containing 25 micrograms of biotin as the free acid are diluted to 250 cc., so that each cubic centimeter contains 0.1 microgram of biotin. One cubic centimeter of inorganic salt solution A is added as the solution is being diluted to volume.

When biotin methyl ester is used, the contents of a 25-microgram ampoule are added to 50 cc. of 0.1 *N* hydrochloric acid and autoclaved for 1 hour at 15 pounds per square inch pressure. One cubic centimeter of inorganic salts solution A is added, the pH is adjusted to 6.6 to 6.8, and the volume is made to 236 cc. Each cubic centimeter, therefore, provides 0.1 microgram of biotin, since 25 micrograms of biotin methyl ester theoretically yield 23.6 micrograms of the free acid on hydrolysis. The use of 0.1 *N* hydrochloric acid is specified above because it was found in this laboratory that autoclaving a neutral aqueous solution of biotin methyl ester for 15 minutes at 15 pounds' pressure did not bring about complete hydrolysis.

p-Aminobenzoic Acid. One hundred milligrams of *p*-aminobenzoic acid are dissolved in about 500 cc. of distilled water and diluted to 1000 cc., so that each cubic centimeter contains 100 micrograms of *p*-aminobenzoic acid.

Nicotinic Acid Solutions. One gram of pure nicotinic acid is dissolved in about 700 cc. of warm distilled water and diluted to exactly 1000 cc. This stock solution should be made up fresh at least every 2 months as a precaution against possible change in potency. Exactly 10 cc. of the above solution are diluted to 1000 cc. to give a 10 microgram per cc. solution of nicotinic acid. This solution is made up fresh each time that the standard (see below) is prepared.

Ten cubic centimeters of the 10 microgram per cc. solution are diluted to 1000 cc. to make the standard solution containing 0.1 microgram of nicotinic acid per cc. This standard is freshly prepared each week.

Commercial c. p. nicotinic acid has been used as received for the primary standard throughout this investigation. A sample taken from a half-used, 115-gram (quarter-pound) bottle was examined for moisture by heating over phosphorus pentoxide at 100° C. and 0.08-mm. pressure for 5 hours. The loss in weight amounted to 0.91 per cent.

Thiamine Chloride, Calcium Pantothenate, and Pyridoxine Solution. A stock solution is prepared in distilled water, and is so diluted as to contain 100 micrograms of each of these vitamins per cc.

The following solutions are made up according to the directions given by Snell and Wright (*13*): riboflavin solution; adenine, guanine, uracil solution; inorganic salts solution A; and inorganic salts solution B.

Stock Cultures and Inoculum. Stock cultures are carried as stabs in an agar medium made up as outlined by Snell and Wright, except that the medium is modified to contain 0.5 per cent dextrose and 0.5 per cent sodium acetate. These cultures are transferred at weekly intervals and at each weekly transfer 3 to 4 extra stab cultures are prepared. Each culture is incubated 24 to 32 hours at 37° C. and then held in the refrigerator until needed.

To grow inoculum for the assay tubes a transfer is made from one of the above stab cultures into a tube containing 10 cc. of the basal medium plus 1 microgram of nicotinic acid. This liquid culture is incubated for 24 hours at 37° C. and the cell suspension so obtained is used for inoculation, either as it is or after diluting with an equal volume of 0.9 per cent saline. This procedure eliminates centrifugation and resuspension of the cells.

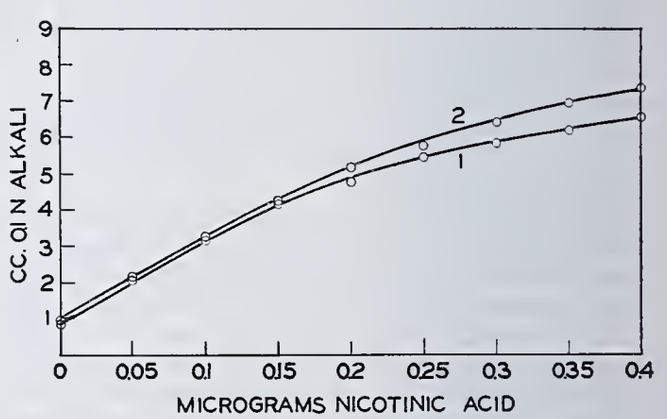

Figure 2. Response to Pure Nicotinic Acid (72 Hours' Incubation)

1. Snell-Wright medium modified to contain 2 per cent glucose
2. Snell-Wright medium modified to contain 0.02 per cent cystine

Inoculum so prepared is not used for growing further inocula by serial transfers in the liquid medium. On the contrary, one should return to a stab culture each time inoculum is to be prepared. One such stab may be used several times if proper precautions are taken to exclude contamination with other organisms.

Preparation of Samples. Dry materials are finely ground, and fresh materials are mechanically homogenized in water—e. g., with a Waring Blendor or Potter-Elvehjem homogenizer (*11*). One gram of material is suspended in 50 cc. of *N* hydrochloric or sulfuric acid, and the suspension is autoclaved 20 minutes at 15 pounds per square inch pressure. The cooled mixture is adjusted to pH 6.6 to 6.8 with *N* sodium hydroxide, and diluted to such a volume that each cubic centimeter contains approximately 0.05 microgram of nicotinic acid. Filtration is unnecessary, but does no harm. Water-soluble materials are dissolved in the acid, autoclaved, neutralized, and diluted as above.

Procedure. To prepare medium sufficient for 100 tubes, the following quantities of the above stock solutions and reagents are required:

Acid-hydrolyzed casein solution	50 cc.
l-Cystine solution	50 cc.
l(-)Tryptophan solution	50 cc.
Anhydrous glucose	20 grams
Anhydrous sodium acetate	20 grams
Biotin solution	2 cc.
p-Aminobenzoic acid solution	1 cc.
Thiamine, calcium pantothenate, pyridoxine solution	1 cc.
Riboflavin solution	2 cc.
Adenine, guanine, uracil solution	10 cc.
Inorganic salts, solution A	5 cc.
Inorganic salts, solution B	5 cc.

These materials are mixed, adjusted to pH 6.6 to 6.8, and diluted with distilled water to 500 cc. Five cubic centimeters of this solution, which is exactly double the concentration of the basal medium, are measured into each tube. Twenty tubes are reserved for blanks and for the standard curve. To these are added 0.0, 0.0, 0.5, 0.5, 1.0, 1.0, 1.5, 1.5, 2.0, 2.0, 2.5, 2.5, 3.0, 3.0, 3.5, 3.5, 4.0, 4.0, 5.0, and 5.0 cc., respectively, of the standard nicotinic acid solution (0.1 microgram per cc.).

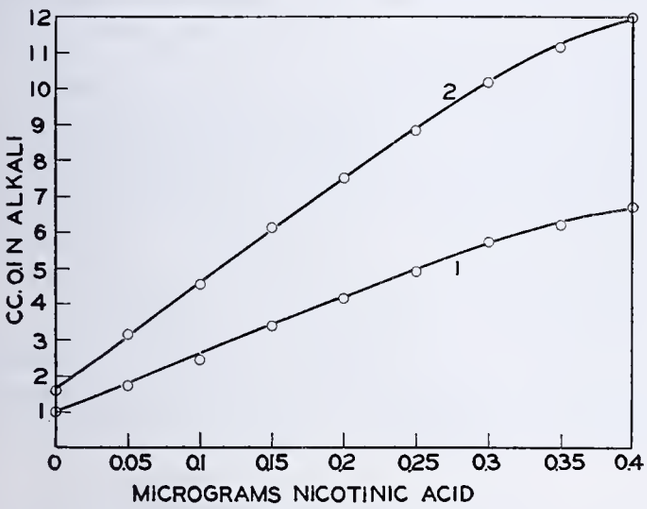

Figure 3. Response to Pure Nicotinic Acid on Present Basal Medium

1. 24-hour incubation
2. 72-hour incubation

The remaining tubes are used for the assay of the test samples. For each sample 8 tubes are used, and to them are added, respectively, 1.0, 1.0, 2.0, 2.0, 3.0, 3.0, 4.0, and 4.0 cc. of the solution prepared as described above. The volume of liquid in each tube is then brought to 10 cc. by the addition of distilled water, the tubes are plugged, autoclaved at 15 pounds per square inch pressure for 15 minutes, cooled to room temperature, and inoculated with 1 drop of inoculum. After 72 hours' incubation at 37° (±1°) C. the contents of each tube are titrated according to the directions given by Snell and Wright (*13*).

Calculation of Results. In evaluating the results of an assay, the agreement between the titration values of duplicate tubes is first noted. These ordinarily do not differ by more than 0.2 to 0.3 cc. of 0.1 *N* alkali, and any tubes which do differ by 0.5 cc. or more are discarded. Such variations probably are attributable either to bacterial contamination or to errors in measuring out the liquids added to the tubes. If the duplicate tubes agree satisfactorily, the titration values are averaged and used as one value in subsequent calculations.

The standard curve is plotted in the usual manner—e. g., curve 2, Figure 3—and from it the nicotinic acid content of each assay tube is determined. Tubes which contain less than 0.04 or more than 0.30 microgram of nicotinic acid are rejected as being outside the useful assay range. The nicotinic acid contents of tubes falling in this range are then converted to micrograms of nicotinic acid per cubic centimeter of test solution, and the agreement at different levels is noted. The final nicotinic acid content of the sample is calculated from the average of at least three such values, none of which deviates more than ±10 per cent from the average.

An alternative procedure for setting up samples and calculating results has been used in this laboratory, in case a great many routine assays are being done by an experienced analyst. In this procedure five tubes are used for each sample, and to them are added 1.0, 2.0, 3.0, 4.0, and 5.0 cc., respectively, of the test solution. The results from at least three of these tubes must fall in the assay range, and must agree to within ±10 per cent to permit calculation of a reliable value for the nicotinic acid content of the sample. The results from tubes which do not satisfy the above requirements are rejected.

Time of Incubation. In practically all this work the tubes have been incubated 72 hours and this period is recommended for general use. However, it is possible to secure usable results in shorter periods, even after as short a period as 24 hours' incubation. A standard curve obtained in this way is shown in Figure 3. It will be noted that this curve is very similar to that obtained in 72 hours by the original Snell-Wright method (Figure 1), but is more nearly linear. Comparative results obtained after 24 and 72 hours' incubation are shown in Table II.

Effect of Cornstarch and Fatty Acids on *L. arabinosus*. Five grams of commercial cornstarch and 50 cc. of 0.1 *N* hydrochloric acid were mixed and autoclaved for 20 minutes at 15 pounds per square inch pressure. The mixture was adjusted to pH 6.6 to 6.8 and diluted to 100 cc. Two other similar preparations were also made in which water and 0.1 *N* sodium hydroxide, respectively, were substituted for the hydrochloric acid. Each solution was assayed for nicotinic acid by the original Snell-Wright method, both directly and after the addition of nicotinic acid equivalent to 5 micrograms per gram of the original starch. The results are shown in Table III.

Samples of oleic, linoleic, stearic, and palmitic acids were also tested for their effect on the nicotinic acid assay. These samples were the same as those used by Strong and Carpenter (*15*) and were tested at the levels found by them to have the

Table II. Comparison of 72-Hour and 24-Hour Assay Results

Samples	24-Hour Assay	72-Hour Assay
	Micrograms per gram	
Defatted corn germ	46.4, 45.7	44.0, 45.5
Raw corn germ	34.8, 35.1	31.8, 30.0
Rice Krispies	108, 107	106, 104
Corn flakes	19.2, 19.5	18.4, 17.6
Solubilized liver extract	102, 101	98.0, 94.0
Wheat middlings	108, 103	104, 99.3
Alfalfa meal	40.8, 39.0	43.2, 38.0
Skim milk powder	10.2, 9.70	8.20, 8.20
Dried yellow split peas	31.8, 32.7	31.8, 33.0
Rice polishings	880, 900	864, 850
Canned green beans	4.70, 4.70	4.50, 4.60

Table III. Recovery of Nicotinic Acid Added to Cornstarch[a]

Volume of Autoclaved Suspension per Tube	Nicotinic Acid Recovered[b] after Autoclaving Starch Suspended in:		
	Water	0.1 *N* HCl	0.1 *N* NaOH
Cc.	%	%	%
1	92	107	102
2	102	102	109
3	109	105	105
4	102	102	107
5	98	102	104

[a] Starch contained 0.4 μg. of nicotinic acid per gram and 5 μg. per gram was added after autoclaving.
[b] Corrected for nicotinic acid content of original starch.

Table IV. Effect of Fatty Acids on Recovery of Known Amounts of Nicotinic Acid

Substances Added per Tube: Fatty Acid Kind	Fatty Acid Amount	Nicotinic Acid	Ratio of Fatty Acid to Nicotinic Acid	Nicotinic Acid Recovered
	Micrograms	*Micrograms*		%
Oleic	48	0.05	960	100
	96	0.10	960	100
	144	0.15	960	100
	192	0.20	960	96
	24	0.05	480	110
	48	0.10	480	100
	72	0.15	480	95
	96	0.20	480	92
Linoleic	40	0.05	800	125
	80	0.10	800	117
	120	0.15	800	115
	160	0.20	800	105
	20	0.05	400	125
	40	0.10	400	122
	60	0.15	400	107
	80	0.20	400	80
Stearic	20	0.05	400	100
	40	0.10	400	100
	60	0.15	400	114
	80	0.20	400	108
	100	0.25	400	106
Palmitic	13	0.05	260	120
	26	0.10	260	110
	39	0.15	260	108
	52	0.20	260	111
	65	0.25	260	106

greatest influence on the riboflavin assay. The method of testing also was the same as that previously used. The results are presented in Table IV.

Discussion

The decision to employ a strong acid to effect extraction of the sample has been based mainly on two considerations. First, the demonstrated existence of combined forms of nicotinic acid which are not measured by *L. arabinosus* (*1, 4, 8*) makes some kind of a hydrolytic procedure imperative if total nicotinic acid is to be determined. Secondly, sodium hydroxide has proved undesirable for general use on account of soap formation with high-fat samples. The data in Tables III and IV indicate that little interference is to be expected from fatty acids in the sample, with the possible exception of linoleic acid. Materials high in this acid should probably be extracted with ether before assay.

If crystalline biotin preparations are used, the casein hydrolyzate will be the only important factor contributing to high blanks. Removal of nicotinic acid from the casein must be made as complete as possible before hydrolysis. Fifty per cent alcohol and 0.1 *N* hydrochloric acid have been suggested for extraction of the casein, but appear to have little if any advantage over 95 per cent alcohol. It is very important that the hydrolyzate be treated with an active charcoal. In the authors' hands Darco G-60 has given the best results.

It is their experience that inocula grown by repeated transfer in the Snell-Wright basal medium are less satisfactory than cells taken directly from the stab culture. This undoubtedly is attributable to deficiencies in that medium. Whether or not the present basal would be more successful in this regard has not been investigated, since the recommended procedure uniformly produces a very active inoculum.

The basal medium recommended in this paper is the result of extensive trials made with many different combinations of ingredients. Additional substances which were tested but appeared not to be beneficial include a folic acid preparation, asparagine, glutamine, ammonium sulfate, ammonium phosphate, glycine, serine, threonine, isoleucine, and various combinations of these materials. A few runs were made in which pyridoxine, thiamine, and riboflavin were omitted from the medium. In agreement with previous observations (*2, 12*) satisfactory results were obtained, and it may well be that these ingredients are superfluous. However, the authors do not feel justified at the present time in recommending that they be left out. The beneficial effect of the increased concentration of sodium acetate is in line with similar observations by Stokes and Martin on *Lactobacillus casei* (*14*).

The main advantages of the modified procedure are the enhanced response of the bacteria to pure nicotinic acid, the linearity of the response, and the greater reliability and reproducibility of the assay results. The bacterial response may be expressed by the average additional acid production elicited by each increment of nicotinic acid. This amounts to 1.40 cc. per 0.05 microgram of nicotinic acid over the range 0.04 to 0.30 microgram for the standard curve given in Figure 3, curve 2. The analogous figure calculated for the same range from the result on the original medium (curve 1, Figure 1) is only 0.86 cc.

Perfectly straight-line standard curves extending to a titration of 10 cc. or over are regularly obtained. The amount of nicotinic acid required to reach the highest point in the linear portion of the curve varies from 0.3 to 0.4 microgram. It is for this reason that the assay range has been given as 0.04 to 0.3 microgram. The range could no doubt be safely extended to 0.4 microgram in those assays where the linear portion of the curve reaches this level of nicotinic acid.

Results show excellent agreement at different levels of sample, and are frequently within ±5 per cent over the entire assay range. The method as here described has been applied routinely to over 500 food samples in this laboratory (*6*), and has been found entirely satisfactory.

Table V. Effect of Oxidizing Agents on Microbiological Assay of Nicotinic Acid in Wheat

Material Assayed	Preliminary Treatment of Extract[a]	Nicotinic Acid Found: Brown *et al.* (*3*)	Nicotinic Acid Found: Present authors
		μg./g.	*μg./g.*
Whole wheat flour (collaborative sample)[b]	None	43.8	40.0
	H_2O_2, HCl	24.75	42.4
	H_2O_2, Lloyd's reagent	23.75	...
	H_2O_2, NaOH	48	47.5
	$KMnO_4$[c]	....	36.8
Wheat bran, 1	None	140	...
	H_2O_2, HCl	138	...
Wheat bran, 2	None	....	188
	H_2O_2, HCl	....	172
	NaOH,[d]	....	288
	NaOH, then H_2O_2, HCl	....	292

[a] Extracts were prepared and treated with Superoxol in acid or alkaline solution exactly according to directions of Brown *et al.* (*3*). Extract of wheat bran 2 was made by boiling for 1 hour with 100 parts of 0.1 *N* HCl.

[b] Obtained from John Andrews—same sample as that used by Brown *et al.* (*3*).

[c] Heated 10 minutes at 100° with excess *N* $KMnO_4$, then decolorized with hydrogen peroxide.

[d] To 20 cc. of 0.1 *N* HCl extract were added 3 cc. of 15 per cent NaOH, mixture was allowed to stand 10 minutes at room temperature and neutralized. This treatment hydrolyzes alkali-sensitive precursor of nicotinic acid which is present in wheat (*1*), and is extracted but not hydrolyzed by 0.1 *N* HCl at 100° (*3*).

A serious criticism has recently been leveled at the microbiological method for the determination of nicotinic acid by Brown, Thomas, and Bina (*3*), who have reported that much lower values were found in various wheat fractions after oxidation with hydrogen peroxide. The authors have repeated their work on whole wheat flour, and are unable to corroborate their findings. A summary of the results is given in Table V. It is apparent that in the authors' hands no variations greater than the experimental error of the method were found after oxidation.

The figure of about 40 micrograms per gram for the whole wheat sample seems to be the correct nicotinic acid content as determined on the extract prepared according to Brown *et al.* There is little doubt that this is much lower than the true value, which is about 64 micrograms per gram (*9*), and that the discrepancy is attributable to incomplete extraction. A similar difference is evident in the case of wheat bran, and is again probably due to the method of extraction used.

The value of 34 micrograms per gram for the nicotinic acid content of wheat germ (*3*, *16*) is probably too low. Assay of a more extended series of wheat germ samples in this laboratory has shown that the germ contains approximately the same amount of nicotinic acid as does whole wheat.

Summary

Modifications in the basal medium proposed by Snell and Wright (*13*) for the microbiological determination of nicotinic acid have been described, as well as a different procedure for growing the necessary inoculum. These variations have resulted in nearly doubling the response of the bacteria to quantities of nicotinic acid in the range 0.04 to 0.3 microgram, and have produced a linear standard curve.

The assay as carried out at present is characterized by a much higher degree of consistency and uniformity than were usually obtained with the method as originally published.

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A Spot Plate for Drop Tests

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At present two common types of spot plates are in use. When a soluble colored reaction product is anticipated, a white spot plate is used. If the reaction product anticipated is a precipitate (other than black, blue, or dark red), a black spot plate is ordinarily selected. During investigations of various drop reactions it became apparent that a combination black and white spot plate would be of advantage, and a plate of the design shown in Figure 1 was ordered. Such a plate is obtained by placing a black glaze on half of an ordinary three-depression white spot plate. The line of demarkation between the black and the white should run exactly through the centers of the three depressions.

The plate as shown is adaptable for either colored solutions or precipitates. Its great advantage lies in the hands of the experienced analyst to whom each intermediate precipitate and color is significant, regardless of the form of the final test product. By observing intermediate colors or precipitates possible interferences due to complex ion formation, competitive reactions, etc., can be anticipated (*1*). A further use is suggested by the work of West and Houtman (*2*), who propose a test for orthophosphate which depends on the formation of a yellow precipitate, best seen over a black surface, which is differentiated from interfering precipitates by converting it to a blue compound which should be observed over a white surface. The combination spot plate has proved invaluable, both in research and in the routine application of drop reactions.

Spot plates of this design can be obtained after the war from the Fisher Scientific Company, Pittsburgh, Penna.

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The top depression contains a clear colorless solution, the center a blue colored compound, and the lower a yellow precipitate. The advantage of the combination plate is best observed by alternately covering the white and black halves.

Figure 1. Combination Black and White Spot Plate

Anhydrous Copper Sulfate in the Kjeldahl Nitrogen Determination

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THE Association of Official Agricultural Chemists (*1*) recommends the use of crystallized copper sulfate (pentahydrate) in the official Kjeldahl-Gunning-Arnold method for the determination of organic nitrogen. Bradstreet (*2*) in a survey of the Kjeldahl method of analysis mentions the use of copper sulfate as a catalyst for the Kjeldahl digestion, but in every case this is the copper sulfate pentahydrate. Niederl and Niederl (*3*) recommend, for the micro-Kjeldahl technique, the use of 1 part of potassium sulfate, 3 parts of copper sulfate pentahydrate, and a small amount of selenium.

This latter procedure, somewhat modified for semimicroquantities, was used for routine determinations of nitrogen in organic compounds. It was found, however, that if the copper sulfate pentahydrate were replaced by anhydrous copper sulfate several advantages were apparent. The anhydrous copper sulfate did not dissolve completely in the sulfuric acid (unlike the pentahydrate) and the fine crystals served as "boiling stones", allowing a much more rapid and smooth digestion, free from all bumping. The size of the flame used was of less importance, since there was no tendency to bump.

Copper sulfate as a catalyst is likewise useful in the distillation of the ammonia as an internal indicator, since the blue color disappears when the solution is made basic, while black cupric oxide is formed.

The semimicromethod of analysis used calls for a sample size of 20 to 35 mg., to which are added 8 ml. of concentrated sulfuric acid, 2 grams of copper sulfate–potassium sulfate mixture and about 20 mg. of powdered selenium. The mixture is digested for 15 to 25 minutes. A finely ground mixture of 1 part of anhydrous copper sulfate and 2 parts of potassium sulfate, with a small amount of selenium is used as catalyst in the semimicro-Kjeldahl analyses run in this laboratory.

Literature Cited

(1) Assoc. Official Agr. Chem., Official and Tentative Methods of Analysis, 4th ed., II, p. 25, 1935.
(2) Bradstreet, R. B., *Chem. Rev.*, 27, 331 (1940).
(3) Niederl and Niederl, "Organic Quantitative Microanalysis", New York, John Wiley & Sons Co., 1938.

Recommended Specifications for Microchemical Apparatus

Correction in the Design of the Dumas Nitrogen Stopcock

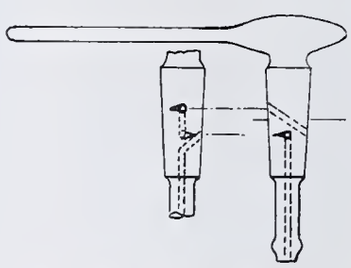

FIGURE 1. INCORRECT

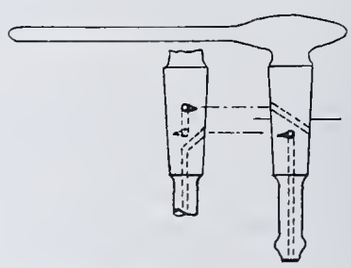

FIGURE 2. CORRECT

IT HAS been called to our attention that the design of the stopcock published in our previous report [IND. ENG. CHEM., ANAL. ED., 13, 580 (1941)] is incorrect, in that it reduces the safe zone of possible leakage from one fourth of the circumference to one eighth or less. To overcome this, we suggest that the stopcock be designed as shown in Figure 2 instead of the previous incorrect procedure shown in Figure 1.

In the incorrect view, the groove on the lower end opening of the straight bore is directed toward the opening of the bent outlet bore. In the correct form, Figure 2, the direction of the groove on the lower end of the straight bore must be away from the opening of the bent bore, so that a safe zone of at least one fourth of the circumference of the stopcock plug remains. The groove of the bent bore may remain as specified.

G. L. ROYER, *Chairman*
H. K. ALBER
L. T. HALLETT
J. A. KUCK, *Secretary*

INDUSTRIAL AND ENGINEERING CHEMISTRY

ANALYTICAL EDITION

PUBLISHED BY THE AMERICAN CHEMICAL SOCIETY • WALTER J. MURPHY, EDITOR

A "P. S." from the Editors

AT THE summer meeting of the Advisory Board of the ANALYTICAL EDITION of INDUSTRIAL AND ENGINEERING CHEMISTRY, attended by the entire membership of the board, a number of suggestions were offered and discussed, designed to improve the service rendered the readers of this publication.

In the future, authors must supply abstracts of their papers if such manuscripts are likely to fill more than one printed page. Further, (1) each article should include a statement of the range of applicability of the suggested method; (2) interferences and interfering substances should be listed; and (3) where possible the precision and accuracy of the method should be given.

We quote from "A Note to Authors", originally published in January, 1937, when the ANALYTICAL EDITION was established on a monthly rather than a bimonthly basis:

> The author should distinguish carefully between precision and accuracy. Briefly but somewhat roughly stated, accuracy is a measure of degree of correctness; precision is a measure of reproducibility. The precision of a result does not necessarily have anything to do with its accuracy; it serves merely as a measure of the duplicability of the procedure in the hands of a given operator. No claim for accuracy should be made unless the author believes that he has satisfactorily established the correct result.

Beginning with this issue new monthly features are to be added—they might more properly be designated as departments. One will be titled "Notes on Analytical Procedures", another, "Book Reviews", and a third will report new scientific apparatus and laboratory equipment. In addition, from time to time news items pertinent to the analytical field will be published, as, for example, one in this issue dealing with priorities on laboratory materials.

At this point it might be well to state the fundamental purpose of the ANALYTICAL EDITION:

1. To publish fundamental chemistry of analysis and analytical methods.
2. To publish applied analytical methods.

It was the consensus of opinion of the entire Advisory Board that long papers in highly specialized fields should not be published; but in their place abstracts or relatively short digests. However, exceptions will be made where such specialized articles apply to fields in which a reasonable number of the readers of this publication are actively engaged and would, therefore, find such articles of great practical value in their work. Here editorial discretion will, of course, be carefully exercised.

In conclusion, the editors wish to point out to authors the limitations now imposed on all publications in the matter of paper supplies. These are particularly serious in the case of scientific publications, like those of the AMERICAN CHEMICAL SOCIETY, which are playing a distinct and useful part in the war effort by supplying valuable technical information.

It has been said that "Brevity is the soul of wit." Indeed, it is much more than that. Conciseness consistent with clarity is asked for by the busy men and women who rightfully are accustomed to look upon the ANALYTICAL EDITION as their "textbook" on analytical chemistry.

Not only are our readers busier than ever before, but so are those who give so willingly of their time and energies in the role of reviewers. This is particularly true as regards the members of the Advisory Board who are constantly being called upon for opinions. Authors should and most of them do prepare manuscripts with great care. There are only very few who do not.

This is YOUR publication. The editors welcome, indeed urge you to submit constructive suggestions and criticisms as they occur to you.

Determination of Wax in Asphalt

RAYMOND L. BETTS AND H. D. WIRSIG[1]

Esso Laboratories, Process Division, Standard Oil Development Company, Elizabeth, N. J.

WAX may materially affect the properties of an asphalt, and the magnitude of the effect will depend upon the specific nature of the wax and the amount present. A small quantity of wax which has a strong tendency to separate in large crystals will be decidedly more noticeable visually than a considerably larger quantity of a microcrystalline or amorphous wax, or one having a greater solubility in the asphalt. Consequently, a reliable procedure capable of evaluating both the character and quantity of wax present in an asphalt is desirable. Recent publications (*2*, *6*) of new methods on the determination of wax in asphalt suggested that the experience at the Esso Laboratories on the same subject might be of interest.

The Holde (*4*) method or the Hubbard (*5*) modification is frequently employed, but it has long been considered unsatisfactory because of the belief that waxes are destroyed by cracking, which results in erroneous conclusions regarding the quantity and nature of the wax present. This belief has been corroborated (*7*, *13*, *15*), and attempts to reduce the error by limiting the time of distillation have not been particularly successful. Because of the limitations of the Holde procedure, other methods (*1*, *9*, *10*, *12*) have been developed but none has proved entirely satisfactory. Maass (*8*), on investigating various methods of treating the asphalt to obtain the oily constituents preparatory to dewaxing in 1 to 1 ether-alcohol or butanone, found that acid treating alone (*12*), treating with adsorbents with or without subsequent acid treating, or acid treating at elevated temperatures (*10*), gave similar results but acid treating followed by distillation (*9*) gave low wax yields.

Because of the conflicting opinions regarding the merits of the various procedures, work was undertaken to study and compare the various methods. The problem consisted of two parts: the selection of a suitable solvent in which residual oils are soluble and waxes are relatively insoluble, and the effect of the several treating methods on waxes and on the recovery of these waxes when added to asphalt in known amounts.

Selection of Solvent

MATERIALS. Paraffin wax, 124° F. (51° C.) melting point.

Petrolatum, 160° F. (71° C.) melting point. This petrolatum had a Tag-Robinson color of 15–18 when liquefied and was obtained from 132° F. (55° C.) melting point petrolatum by clay treating in 4 volumes of naphtha and allowing to settle at 70° F. (21° C.).

Ether, Squibb, U. S. P.

Absolute alcohol

A. S. T. M. naphtha

Trichloroethylene, commercial

Methyl-*n*-butyl ketone, commercial

sec-Butyl acetate, boiling point 111–113° C.

Colombian distillate cylinder oil, 188 viscosity S. S. U. at 210° F. (99° C.)

Pennsylvania bright stock, 143 viscosity S. S. U. at 210° F. (99° C.). This stock was obtained by centrifuging Pennsylvania cylinder oil in 3 volumes of naphtha at −4° F. (−20° C.).

EXPERIMENTAL. Critical solution temperatures of waxes were obtained by weighing the wax in a bottle equipped with a stopper holding a thermometer. The correct volume of solvent was added and the mixture warmed until complete solution was obtained. The solution was then cooled slowly with frequent shaking and the temperature at which the first sign of turbidity appeared was taken as the point of limiting solubility of the wax in

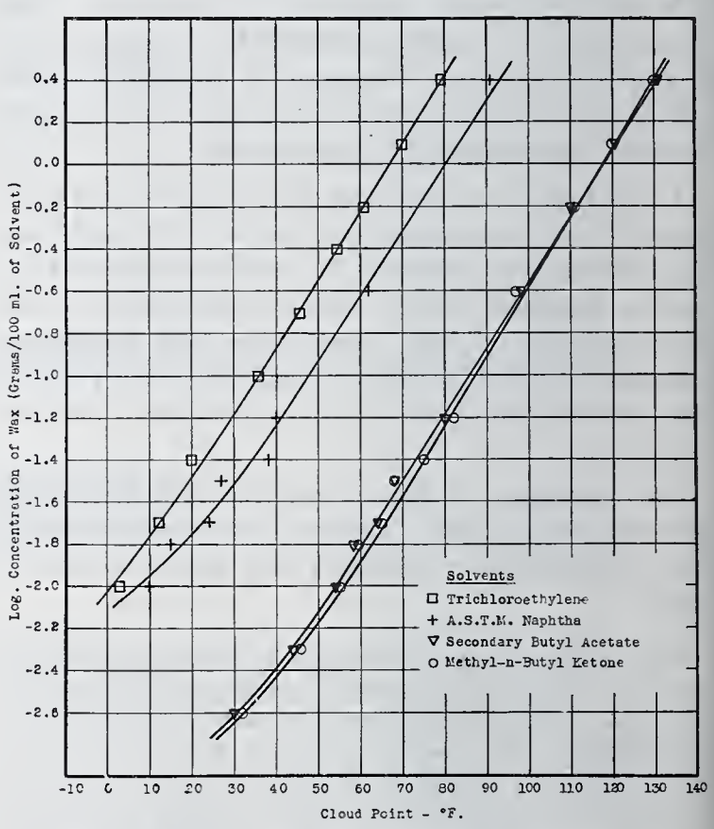

FIGURE 1. RELATION BETWEEN TEMPERATURE AND SOLUBILITY OF 124° F. (51° C.) MELTING POINT WAX IN VARIOUS SOLVENTS

[1] Present address, U. S. Army.

TABLE I. CLOUD POINTS OF SOLUTIONS OF WAXES IN VARIOUS SOLVENTS

Wax Melting Point	Solvent Used	Concentration of Solution (Grams per 100 Ml. of Solvent)														
		2.5	1.25	0.625	0.4	0.25	0.20	0.10	0.0625	0.04	0.032	0.020	0.016	0.010	0.005	0.0025
° F.		° F.	° F.	° F.	° F.	° F.	° F.	° F.	° F.	° F.	° F.	° F.	° F.	° F.	° F.	° F.
124	*sec*-Butyl acetate	83	75	66	..	55	..	...	33	30	..	20	..	8	−8	..
	Methyl-*n*-butyl ketone	82	74	66	..	55	..	...	35	30	..	20	..	9	−8	..
	3 to 1 ether-alcohol	79	71	64	..	51	..	...	...	25	..	16	..	5	−6	..
	A. S. T. M. naphtha	46	...	...	26	19	..	...	−3	−11	..	..	..	..	...	..
	Trichloroethylene	40	31	23	15	..	8	−2	...	−14	..	..	..	..	...	..
160 (petrolatum)[a]	*sec*-Butyl acetate	131	121	110	..	98	..	...	80	..	68	64	59	54	44	30
	Methyl-*n*-butyl ketone	130	120	111	..	97	..	...	82	75	68	65	58	55	46	32
	A. S. T. M. naphtha	91	...	...	..	62	..	...	40	38	27	24	15	10	...	..
	Trichloroethylene	79	70	61	54	..	46	36	...	20	..	12	..	3	...	..

[a] Solutions of 160° F. M. P. petrolatum in 3 to 1 ether-alcohol could be obtained only at very low concentrations.

the solvent. Cloud points of the cylinder stocks and of wax-oil mixtures in each solvent were obtained in a similar manner, the weight of oil or wax plus oil being 2.5 grams per 100 ml. of solvent in all cases.

Results. The cloud points of solutions of waxes in various solvents, containing the waxes in varying concentrations, are shown in Table I and illustrated graphically in Figures 1 and 2. These curves indicate that there is a semilogarithmic relationship between temperature and wax solubility at temperatures above 50° F. (10° C.) as reported in the literature, but at lower temperatures there is a distinct deviation from linearity. There are no appreciable differences in the solubility of 124° F. (51° C.) melting point wax in 3 to 1 ether-alcohol, methyl-*n*-butyl ketone, and *sec*-butyl acetate, but 160° F. (71° C.) melting point petroleum is much less soluble in the first solvent than in the others. All three solvents show much less solvent power for wax than trichloroethylene or A. S. T. M. naphtha. Although solubility data using 1 to 1 ether-alcohol were not obtained with these particular wax samples, other experiments showed that waxes are even less soluble in this mixture than in any of the other solvents.

Cylinder oils (Table II) are not particularly soluble in trichloroethylene and relatively insoluble in 1 to 1 ether alcohol, showing that the latter solvent is not suitable for the separation of wax from oil. The other solvents studied show comparatively high solvent power for oils, and differ from one another only slightly.

Table II. Miscibility Temperatures of Dewaxed Oils in Various Solvents in 2.5 Per Cent Concentration

Solvent	143 S. S. U. Viscosity 210° F. Penn. Bright Stock		Colombian 188 S. S. U. Viscosity 210° F. Distillate	
	° F.	° C.	° F.	° C.
Methyl-*n*-butyl ketone	−30	−34.4	−40	−40.0
3 to 1 ether-alcohol	−40	−40.0	−35	−37.2
sec-Butyl acetate	−25	−31.7	−30	−34.4
A. S. T. M. naphtha	−25	−31.7	−40	−40.0
1 to 1 ether-alcohol	25% insoluble at room temperature		+30	− 1.1
Trichloroethylene	20	− 6.7	+20	− 6.7

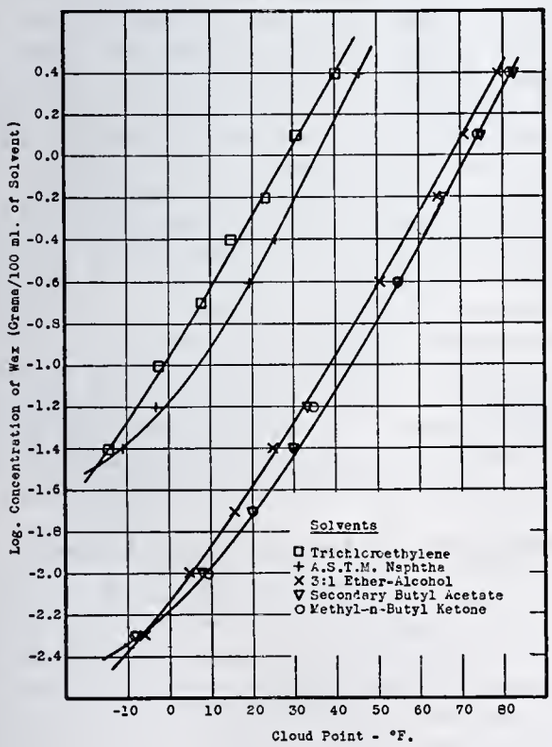

Figure 2. Relation between Temperature and Solubility of 160° F. (71° C.) Melting Point Petrolatum in Various Solvents

Table III. Dewaxing Differential

	124° F. M. P. Wax Versus Colombian distillate	124° F. M. P. Wax Versus Penn. bright stock	160° F. M. P. Petrolatum Versus Colombian distillate	160° F. M. P. Petrolatum Versus Penn. bright stock
	° F.	° F.	° F.	° F.
Methyl-*n*-butyl ketone	122	112	170	160
3 to 1 ether-alcohol	114	119	...	...
sec-Butyl acetate	113	108	161	156
A. S. T. M. naphtha	86	71	131	116
Trichloroethylene	20	20	59	59

The "dewaxing differentials" which represent the differences in degrees Fahrenheit between the cloud points of solutions of the wax and of the oil, each present in a concentration of 2.5 grams per 100 ml. of solvent, are recorded in Table III. Methyl-*n*-butyl ketone, *sec*-butyl acetate, and 3 to 1 ether-alcohol exhibit relatively high dewaxing differentials of approximately the same magnitude and appear to be the most effective solvents for dewaxing oil-wax mixtures. Of these three, the ether-alcohol is the least desirable mixture because of its high volatility, which makes it difficult to obtain solutions of high melting waxes in reasonable concentration. The presence of cylinder oils does not appreciably affect wax solubility, since the cloud points of solutions of oil-wax mixtures (Table IV) are substantially the same as those obtained when the wax alone was present in the same solvent in the same concentration (values shown in brackets).

These results indicated that either methyl-*n*-butyl ketone or *sec*-butyl acetate would be satisfactory for separation of wax from oil.

In order to confirm this conclusion, varying quantities of 124° F. (51° C.) melting point wax were mixed with sufficient 188 S. S. U. viscosity at 210° F. (99° C.) Colombian distillate to give 1.0-gram samples of wax plus oil. The mixtures were then

Table IV. Cloud Points of Solutions of Oil-Wax Mixtures in Various Solvents

(In all cases solutions contained 2.5 grams of oil plus wax per 100 ml. of solvent)

Wax in Mixture	Cloud Points[a] 124° M. P. Wax			160° M. P. Petrolatum		
%	° F.	° F.	° F.	° F.	° F.	° F.
	Solvent, Methyl-*n*-Butyl Ketone					
0		−40			−40	
5	43		(45)	82		(87)
10	53		(55)	93		(96)
25	64		(66)	110		(111)
50	73		(74)	120		(120)
100		64			130	
	Solvent, *sec*-Butyl Acetate					
		−30			−30	
5	42		(45)	80		(86)
10	52		(55)	92		(96)
25	65		(66)	108		(110)
50	71		(74)	120		(121)
100		83				
	Solvent, 3 to 1 Ether-Alcohol					
0		−35			−35	
5	42		(41)	..	..	...
10	52		(51)	..	..	...
25	64		(63)	..	..	...
50	71		(71)	..	..	...
100		79				

[a] Figures in parentheses are cloud points of same wax in same concentration in solvent, but free from oil.

Table V. Wax Recovery

(From mixtures of 124° F. M. P. wax and 188 S. S. U. viscosity at 210° F. Colombian distillate in methyl-*n*-butyl ketone)

Wax in 1.0 Gram of Mixture	Recovered Wax	
	Yield	Melting point
Gram	%	° *F.*
1.00	98	125
0.50	101	123
0.25	108	127
0.10	98	127
0.05	101	125

Table VI. Effect of Adsorbent on Wax Yield by the Schwarz Method

Crude Oil Sample	Nuchar		Bone Char (Fat-Free)		Filtrol
	Wax	Extraction time	Wax	Extraction time	Wax
	%	*Hours*	%	*Hours*	%
1	0.19	18	2.12	24	7.55
2	0.22	18	2.85	5	11.45
3	0.98	4	5.41	5	11.03
4	0.17	18	3.21	24	9.27

dewaxed in 10 ml. of methyl-*n*-butyl ketone at −4° F. (−20° C.) by a procedure essentially the same as that described by Hubbard (*5*).

The results obtained are given in Table V and indicate that waxes can be recovered substantially unchanged with a precision of ±5 per cent.

Schwarz Method

Following the selection of solvents suitable for the separation of wax from oil, an examination was made of the Schwarz (*10*) method to determine the effect of the nature of the adsorbent on wax yield and the effect of strong sulfuric acid on refined waxes at elevated temperatures.

Samples (10 grams) of various waxy crudes were digested at 350° F. (177° C.) with 96 per cent sulfuric acid until free from the odor of sulfur dioxide. The resulting coke was mixed with 35 grams of fat-free bone char, as recommended, or of Nuchar, and extracted in a Soxhlet with 400 ml. of 86° Bé. naphtha until a few drops of extract showed practically no evidence of oil after evaporation on a watch glass. In other cases, the coke was placed on a Gooch filter 4 cm. in diameter containing 10 grams of filtrol which had been dried for 16 hours at 225° F. (107° C.) and washed with 250 ml. of 86° Bé. naphtha. In all cases the resulting oils, free of naphtha, were dewaxed in 20 volumes of 3 to 1 ether-alcohol by the Hubbard procedure.

It is evident (Table VI) that the nature of the adsorbent has a very marked effect on wax yield and that excessively long periods of extraction would be necessary to separate the wax completely from the adsorbent.

The higher yields obtained when the coke was washed on a Super-Filtrol bed suggests that this procedure is preferable. Later experience, however, demonstrated that much larger quantities of naphtha are required for washing than are specified here.

The effect of strong acid on wax was studied by digesting 1.0-gram samples of 124° F. (51° C.) melting point paraffin wax and 165° F. (74° C.) melting point petrolatum in 90 per cent sulfuric acid at 400° F. (204° C.). [This wax was obtained by crystallizing 160° F. (71° C.) melting point petrolatum from 4 volumes of *sec*-butyl acetate at 30° F. (−1° C.).] It was found (Table VII) that the waxes were readily attacked under conditions comparable to those employed in the Schwarz procedure, particularly in the case of the lower melting wax. In view of these results it appears that the Schwarz method is unreliable for determining wax contents of oils.

Adsorbent Method

Claims are made in the literature (*1, 8, 14*) that wax in asphalts can be obtained by dewaxing the oily constituents remaining after treatment of the asphalt with adsorbents. The adsorbent method has been used in these laboratories to determine the resin contents of asphalts and it has been found that the quantity of adsorbent required to give oils of a given color varies considerably with the nature of the asphalt. Since the quantity and character of the adsorbent were also found to have a marked effect on wax yield by the Schwarz method, tests were made to determine whether straight waxes could be recovered after treatment with activated clay in the absence of asphalt.

In order to determine the minimum quantity of adsorbent to use, samples of Venezuelan heavy flux were dissolved in 40 volumes of 86° Bé. naphtha, and after separation of asphaltenes were treated with varying amounts of Super-Filtrol. About 8 to 10 grams of clay per gram of asphalt were required to yield a solution of 20 Tag-Robinson color. Accordingly, 1-gram samples of 124° F. (51° C.) melting point wax and 160° F. (71° C.) melting point petrolatum (equivalent to 5 per cent by weight of a 20-gram asphalt sample) were treated in the absence of asphalt in 800 ml. of 86° Bé. naphtha with 160 grams of Super-Filtrol. The clay-wax mixtures were then extracted in Soxhlet extractors for varying periods of time and the quantity and melting points of the waxes recovered were determined. A sample of Venezuelan heavy flux was also treated in a similar manner.

Table VII. Effect of Strong Sulfuric Acid on Wax

	Waxes Treated with 1 Cc. of 90% H_2SO_4 per Gram of Wax	
	124° F. M. P. Wax	165° F. M. P. Petrolatum
Wax recovery, %	41.2	84.9
M. P. of recovered wax, ° F.	132	163

In the case of the recovery of the waxes from the Super-Filtrol remaining from the treatment of their naphtha solutions, the paraffin wax could be effectively recovered by extracting for 9 hours, although a small amount of the higher melting waxes remained in the clay. On the other hand, only about 55 to 60 per cent of the high melting petrolatum could be recovered in a similar time and it was by no means completely separated after 43 hours' extraction, the higher melting portions being the most difficult to remove. In the extraction of the Super-Filtrol after decolorization of the naphtha solution of the asphalt, it was found that the oily constituents were substantially, but not sharply, removed after extracting the clay for 6 hours (Table VIII). The products obtained on longer periods of extraction were of resinous nature, becoming increasingly hard and dark in color. It is apparent from these results that the extraction time of 6 to 16 hours, commonly employed in the adsorbent method of asphalt analysis, would indicate lower melting and smaller amounts of wax than are actually present. Sufficient extraction to remove all wax is impracticable and in addition it is difficult to establish a well-defined end point.

Richardson or Acid-Treating Method

A method which has been extensively used for the determination of wax in asphalts (*12*) involves the use of sulfuric acid to remove resins before dewaxing. In view of the fact that hot sulfuric acid readily attacks and alters the properties of waxes, it appeared possible that cold acid may also have some effect. It was found, however, that 1-gram samples of 124° F. (51° C.) melting point paraffin wax and 160° F. (71° C.) melting point petrolatum dissolved in 500 ml. of 86° Bé. naphtha are unaffected when repeatedly treated with

Table VIII. Effect of Super-Filtrol (Activated Clay) on the Recovery of Waxes

Venezuelan Heavy Flux		124° F. M. P. Paraffin Wax (Alone, No Asphalt)			160° F. M. P. Petrolatum (Alone, No Asphalt)		
Extraction period	Oily constituents	Extraction period	Recovery	M. P. of recovered wax	Extraction period	Recovery	M. P. of recovered wax
Hours	*Wt. % of asphalt*	*Hours*	*Wt. % of original*	*° F.*	*Hours*	*Wt. % of original*	*° F.*
0–6	48.20[a]	0	59.9[b]	119	0	26.6[b]	108
6–16	0.97	0–9	36.2	132	0–8	28.8	143
16–30	1.05	9–19	0.6	...	8–21	13.3	157
30–35	0.41	...	...	...	21–30	5.8	161
					30–43	5.6	165
Total yield	50.63	...	96.7	...	...	80.1	...

[a] Combined material obtained by decantation and subsequent extraction of clay for 6 hours.
[b] Material obtained from decanted solution prior to extraction of clay.

Table IX. Determination of Dewaxing Conditions Suitable for Quantitative Recovery of Waxes Added to Oily Constituents

Dewaxing Solvent	3 to 1 Ether-Alcohol	Ether			*sec*-Butyl Acetate				
Wax mixture, ml. of solvent per gram of oil	10	10	10	10	10	10	20	10	20[a]
Dewaxing temperature, ° F.	−4	−20	−4	−4	16	−4	−4	−4	−4
Added wax, melting point, ° F.	124	124	124	165	124	124	124	165	165
Added wax, % of oils	15.5	14.8	14.6	13.7	13.9	15.2	15.2	15.0	25.8
Recovered wax, % of oils	41.7	19.9	16.8	13.2	17.0	22.2	16.6	15.4	22.6
Recovered wax, % of added wax	269.0	134.5	115.1	96.4	122.3	146.1	109.2	102.7	87.6
Recovered wax, melting point, ° F.	108	121	122	147	123	123	123	158	158

[a] Wax recrystallized.

98 per cent sulfuric acid at room temperature. During the test it was observed that the high melting petrolatum was not particularly soluble in 86° Bé. naphtha and tended to separate and collect at the interface during acid treating. Consequently in all subsequent work, acid treating was carried out at 120° to 130° F. (44° to 54° C.) using as asphalt solvent a 70° A. P. I. naphtha having a boiling range of 140° to 210° F. (60° to 99° C.).

In preliminary tests of this method it was found that the minimum acid treatment required varied with the nature and source of the asphalt. To be certain that the acid reaction was complete, acid treating and separating the sludge were continued until there was no change in the volume of acid after shaking with the solution. In washing the naphtha solution free from acid, emulsification difficulties were experienced which were overcome to a considerable degree by washing with a solution of 25 grams of sodium sulfate in a mixture of 300 ml. of alcohol and 700 ml. of water. The resulting naphtha-free oils were relatively insoluble in methyl-*n*-butyl ketone, although earlier studies indicated that this solvent was satisfactory for the separation of waxes from cylinder oils.

Since the particular sample of methyl-*n*-butyl ketone employed did not prove satisfactory, a study was made of the efficiency of several other solvents for dewaxing oily constituents from asphalts. Oils obtained by acid-treating Venezuelan heavy flux were first dewaxed by the selected solvent, then weighed amounts of wax were added to the resulting oils, and the oil-wax mixtures were again dewaxed. The high yield and low melting point of the product obtained using 3 to 1 ether-alcohol (Table IX) indicated the relative insolubility of heavy oils in this solvent. Ether employed in a similar dilution (10 ml. of solvent per gram of oil-wax mixture) at −4° F. (−20° C.) effected a satisfactory separation of 124° F. (51° C.) melting point wax from oil. This solvent is not convenient to use because of its high volatility which makes it difficult to obtain complete solution of oil and wax if there is an appreciable quantity of high melting waxes present. *sec*-Butyl acetate in the ratio of 20 ml. of solvent per gram of oil-wax mixture appeared to be a satisfactory dewaxing medium at −4° F. (−20° C.). The high wax yield at a ratio of 10 to 1 indicated the presence of oils, although this was not reflected particularly in the melting points of the products. It was found inadvisable to dewax at temperatures above 16° F. (−8.9° C.) as the wax loss due to solubility in the solvent would be greater than with twice the solvent at the lower temperature.

In order to test the suitability of the acid-treating method using *sec*-butyl acetate as the dewaxing solvent for obtaining the wax contents of asphalts, wax determinations were made on Venezuelan heavy flux containing known amounts of added waxes. Twenty-gram samples of the asphalt alone and containing 2, 5, and 10 per cent by weight of 124° F. (51° C.) melting point wax or 165° F. (74° C.) melting point petrolatum were refluxed with 200 ml. of the 70° A. P. I. naphtha, filtered, and the asphaltenes washed with 300 ml. of hot naphtha (120° F.) (49° C.). The solution of petrolenes, maintained at approximately 120° F. (49° C.), was then acid-treated, neutralized, and freed from naphtha, yielding oily constituents which were dewaxed in 20 ml. of *sec*-butyl acetate per gram of oil-wax mixture, at −4° F. (−20° C.).

The results of preliminary determinations which were obtained in the development of the procedure, are given in Table X and show that over the range of 2 to 10 per cent of added waxes both types of waxes can be recovered (total wax less the wax content of the original asphalt) in substantially their original form within 0.5 per cent and usually within 0.2 per cent on the asphalt. The wax originally present in the asphalt did not appear to influence the melting point of the added wax. In contrast with these data are those (Table X) obtained by the Hubbard method on similar materials. The significantly lower wax yield and the very great change

Table X. Recovery by Acid Treating, Aluminum Chloride Treating, and Hubbard (Holde) Methods of Waxes Added to Asphalts

Method	Asphalt Used	Added Wax: Melting point	Added Wax: Asphalt	Wax Yield of Asphalt	Melting Point	Recovery[a] of Added Wax
		° F.	*Weight %*	*Weight %*	*° F.*	*Weight %*
Acid	Venezuelan heavy flux	...	0	2.41	133	...
		124	2	4.83	123	2.42
		124	5	7.73	123	5.32
		124	10	12.44	123	10.03
		165	2	4.27	160	1.86
		165	5	7.45	160	5.04
		165	10	12.17	161	9.76
Hubbard	Venezuelan heavy flux	...	0	1.12	121	...
		124	5.26	5.44	114	4.32
		165	5.26	5.20	122	4.08
Aluminum chloride	Colombian, 31/40 Penn.	...	0	3.06	120	...
		165	5.26	8.11	161	5.05

[a] Total wax yield less wax in original asphalt.

in melting point when high melting petrolatum was present, are further indications that the waxes present in asphalts may be greatly altered by the Hubbard (Holde) method. When the specified 1 to 1 ether-alcohol mixture is replaced by *sec*-butyl acetate as the dewaxing solvent in the Hubbard procedure, the wax yield is even lower (Table XI). There is little doubt, therefore, that the Holde procedure will not give reliable indications of either the quantity or melting points of waxes in asphalts.

Since the acid-treating method appeared to be without deleterious effect on waxes, it was employed for the determination of waxes in asphalts from numerous crudes. In the course of the work difficulty was experienced with emulsions and frequently the wax product obtained was very dark in color and sometimes did not have a satisfactorily distinct melting point. Consequently, experiments were carried out to determine whether treatment of petrolenes with aluminum chloride instead of sulfuric acid would reduce these difficulties. Preliminary tests indicated that emulsification was reduced, waxes were unaffected by this reagent at the temperature of treatment, and wax added to asphalts could be recovered equally well, as illustrated by the Colombian 31/40 penetration asphalt in Table X.

Table XI. Effect of Dewaxing Solvent on Wax Contents by the Hubbard (Holde) Method

Wax added to asphalt	None		5.26% of 124° F. M. P.	
Dewaxing solvent	1:1 ether alcohol	*sec*-butyl acetate	1:1 ether-alcohol	*sec*-butyl acetate
Wax yield, weight %	0.74	0.61	4.43	3.05
Melting point, ° F.	122	...	115	117

Wax Contents of Asphalts by Acid and Aluminum Chloride Treating Methods

Procedure. A 20-gram sample of asphalt, weighed to the second decimal place, is spread over the walls of a 500-ml. balloon flask, heat being applied if necessary. The asphalt is gently refluxed with 200 ml. of 70° A. P. I. naphtha until all tarry material has disappeared, then cooled slightly and mixed carefully with 5 grams of Filter-Cel (diatomaceous earth). The hot mixture is filtered with the aid of suction through a percolating tube 5 cm. (2 inches) in diameter and 35 cm. (14 inches) in length and containing a cotton plug covered with a 1.25-cm. (0.5-inch) layer of Filter-Cel. The flask and residue on the filter are washed with portions of hot naphtha (about 120° F., equivalent to 48° C.), totaling 200 ml.

The solution of petrolenes in naphtha is transferred to a 1000-ml. separatory funnel, hot naphtha being used to rinse the suction flask, and the separatory is placed in a steam-heated oven maintained at 125–130° F. (51–54° C.). When temperature equilibrium is reached, the solution is treated with 10 or 20 ml. of 98 per cent sulfuric acid, and the sludge is allowed to settle out. After the sludge has been withdrawn, the acid treatment is repeated until the volume of recovered acid is unchanged after treatment. The solution is then neutralized with 5° Bé. caustic soda and washed neutral to litmus, using a solution containing 25 grams of sodium sulfate in a mixture of 300 ml. of alcohol and 700 ml. of water, the temperature being kept at 120° to 130° F. (49° to 54° C.).

When anhydrous aluminum chloride is used, the solution of petrolenes obtained as above is transferred to a 1000-ml. balloon flask and refluxed for 0.5 hour with 10 grams of the solid reagent. The mixture is then allowed to stand for 15 to 30 minutes at 120° F. (51° C.), when the supernatant solution is decanted to another flask, and the hot naphtha used to rinse the sludge is added thereto. The aluminum chloride treatment is repeated until there is only a slight formation of a red-colored sludge. Finally the solution is washed in a 1000-ml. separatory at 120° F. (49° C.) with 50 per cent alcohol until the wash is neutral.

The neutral solution from either the acid or aluminum chloride treatment is dried in a 1- or 2-liter balloon flask by refluxing in a Dean and Stark apparatus until there is no further increase in the volume of water in the collecting tube, and then concentrated to 50 ml. by distillation. The concentrate together with small portions of hot naphtha used to rinse the flask is transferred to a tared 250-ml. beaker. The remaining naphtha is removed by careful evaporation on the steam bath and the resulting oily constituents are heated in an oven at 230° F. (110° C.) for 0.5 hour, then cooled and weighed to the second decimal place.

Dewaxing. The dewaxing equipment used is similar to that employed by Hubbard, except that the funnel is made of brass rather than glass. This brass funnel, illustrated in Figure 3, is of the Büchner type, and is fitted with a long barrel, *d*, threaded at one end, by means of which it can be attached to the funnel cone, *a*, in such a manner as to fit tightly against the perforated filter plate, *b*. To prepare the funnel the lower end of the filter tube is stoppered and sufficient dewaxing solvent added to fill the tube until the perforated filter plate placed in position *e* is just covered. A piece of snugly fitting No. 10 duck

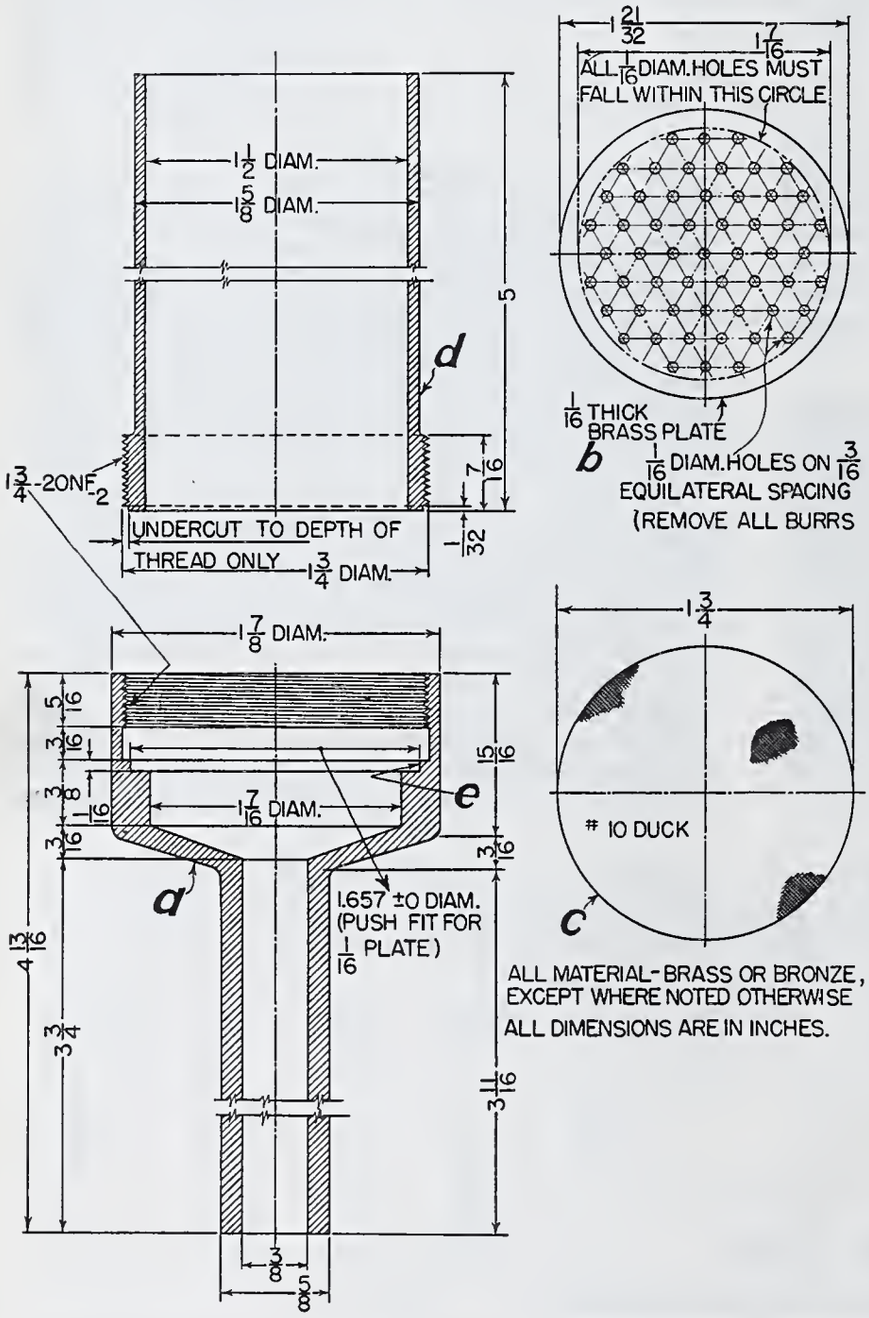

Figure 3. Wax Filter

filter cloth, *c*, is then placed on the plate and the funnel barrel is inserted and screwed down tightly, thus firmly clamping the filter cloth in position. A 0.6-cm. (0.25-inch) layer of Filter-Cel is placed on the cloth and moistened with 10 ml. of *sec*-butyl acetate, and the assembled tube is placed in a bell-shaped funnel which serves as a cooling bath.

A 3 ± 0.01-gram sample of the oily constituents is dissolved in 25 ml. of *sec*-butyl acetate in a 50-ml. beaker, and warmed on a hot plate sufficiently to obtain a clear solution. The resulting solution is transferred to the prepared filter tube, using 25 ml. of warm *sec*-butyl acetate to rinse the beaker. After the contents of the tube have been gently stirred, the tube is stoppered with a cork through which passes a thermometer adjusted so that its bulb is immersed in the solution. Alcohol is now added to the bell-shaped funnel until it is within an inch of the funnel top, and three or four stoppered test tubes, each containing 20 ml. of *sec*-butyl acetate, are immersed therein. The bath is chilled by the addition of dry ice and, during chilling, the contents of the tube are stirred occasionally until a temperature of −4° F. (−20° C.) is reached, which is maintained constant for 15 minutes. The cork is removed from the bottom of the filter tube, filtration is then carried out with the aid of suction, and the wax cake is stirred vigorously with a portion of prechilled solvent and again filtered.

Washing is repeated until the issuing liquid is colorless, then the cold alcohol is removed from the bath, and the apparatus allowed to come to room temperature. The filter assembly is removed, dried with a towel, and attached to a 250-ml. suction flask. Hot 70° A. P. I. naphtha is added to the tube, stirred vigorously, and withdrawn with suction. Then naphtha (20 ml.) is added to the tube and, after the side arm of the suction flask has been stoppered, the combination is placed on a hot plate and refluxed until condensate appears at the funnel top. After cooling slightly, filtration is carried out with suction and the tube rinsed with 70° A. P. I. naphtha. The filtrate thus obtained is evaporated in a tared crystallizing dish on a steam bath and the resulting wax is heated at 230° F. (110° C.) to constant weight, ±0.001 gram.

Wax melting points are obtained by the rotating thermometer method. A small quantity of wax is placed on the bulb of a thermometer and warmed until the wax is in a molten condition, the thermometer is inserted in a test tube to protect it from air currents, then gently rotated, and the temperature at which solidification occurs is recorded as the melting point.

Table XII. Comparison of Wax Contents of Various Asphalts by Aluminum Chloride and Acid-Treating Procedures

Asphalt	Aluminum Chloride Method				Acid Method			
	A		B		A		B	
	Yield *Weight* %	Melting point ° F.	Yield *Weight* %	Melting point ° F.	Yield *Weight* %	Melting point ° F.	Yield *Weight* %	Melting point ° F.
Kern River, 127° F. S. P.	0.88	139	0.82	139	1.15	140	1.01	142
Mexican, 31/40 Penn.	0.95	138	0.80	141	1.79	134	1.82	135
Venezuelan, 180/200 Penn.	1.00	137	1.04	142	1.96	126	1.95	127
Colombian, 31/40 Penn.	3.03	126	3.08	126	5.63	122	5.33	122
Talco, 41/50 Penn.	3.39	163	3.03	162	6.34	153	6.54	154
Mid-continent, binder C	5.12	148	4.82	149	6.34	136	6.71	142
Sumatra residuum (high softening point)	4.44	148	...	...	5.57	149	5.53	147
Sumatra residuum (low softening point)	11.47	161	11.10	165	12.70	161	13.52	161

Discussion and Results. The relatively large asphalt sample (20 grams) is recommended to ensure sufficient oil-wax mixture for the dewaxing step. Some high melting asphalts may contain as little as 10 per cent of oily constituents. Maintenance of an elevated temperature during treating and filtering to obtain resin-free oils is probably not necessary in most cases, but it was found essential when analyzing asphalts containing appreciable quantities of high melting waxes—e. g., wax determinations on a Sumatran asphalt carried out at room temperature resulted in widely deviating results and indicated that the waxes of higher melting point were lost. These losses were avoided by conducting the test at elevated temperatures. For the same reasons it was found necessary to reflux with the naphtha in the filter assembly when dissolving the wax cake from the filter after dewaxing. The brass filter tube was employed because it is durable, it is a good conductor of heat, and its design permits the wax cake to be broken up and mixed with the solvent, thus ensuring complete washing without in any way diminishing the efficiency of the filter bed.

The results obtained in duplicate on a variety of asphalts by the sulfuric acid and aluminum chloride treating methods are shown in Table XII. It is apparent that either method would yield reproducible results and would serve to classify asphalts with respect to both quantity and melting point of waxes present. The acid method, however, gave higher yields of wax, sometimes nearly twice as great, and the melting points were nearly always lower than those obtained by the aluminum chloride method. Preliminary work on acid treating indicated that strong sulfuric acid was prone to react with wax and resins, giving sulfonated products which caused high wax yields and the production of very dark colored materials that did not possess sharp melting points. It was also found difficult to obtain what appeared to be resin-free oils from some asphalts by treating with 96 per cent sulfuric acid. On treating with aluminum chloride no particular difficulty was experienced with any of the asphalts examined. For these reasons the aluminum chloride method has been chosen for the wax determinations in these laboratories. Both methods indicate that asphalts contain larger amounts of waxes having higher melting points than can be obtained by the Holde procedure.

Since completing this work two new methods have been proposed for the determination of wax in asphalt. One method (*2*) uses isobutyl alcohol at 130° F. (54° C.) to separate resins from oil and wax. This may be satisfactory when the waxes present are low melting, but if they have a melting point approaching 160° F. (71° C.) it is improbable that they would remain in solution at the temperature specified; consequently the method would not appear to be generally applicable. The second proposed method (*6*), using propane for deasphalting and deresining and hexone for dewaxing the resulting oil-wax mixtures, appears to be sound. The limited amount of data cited does not indicate whether the method is generally applicable to various types and grades of asphalts, but the results are in harmony with those obtained by the acid or aluminum chloride methods herein described in showing that waxes in asphalts are higher melting and are present in greater quantity than indicated by the Holde method.

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Viscosity of Solutions in Branched-Chain Paraffins

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Solutions of an alkyd-type resin, and of a natural and two substitute rubbers, at high concentrations in two pentanes, four hexanes, five heptanes, and two octanes show much higher absolute and relative viscosities in the branched paraffins than in the corresponding normal paraffins. An arbitrary method of predicting the viscosity of the resin solutions in branched paraffins has been devised. Large differences between solution viscosities suggest a rough method of hydrocarbon analysis.

A PREVIOUS paper (*3*) showed that solutions of certain resins and linear polymers in isoparaffinic hydrocarbon solvents exhibited much higher absolute and relative viscosities than solutions in corresponding normal paraffins. Comparisons were limited to alkyd-type resins and polybutenes, dissolved in *n*-hexane, neohexane, *n*-heptane, and triptane. Measurements have now been extended to include two pentanes, four hexanes, five heptanes, two octanes, and normal nonane and decane. Physical properties of these hydrocarbons are shown in Table I. Solutes include milled samples of natural and substitute rubbers, and a nonphthalic alkyd resin. Most of the measurements were made with the resin solute.

Viscosity Measurement

The technique used previously was employed in making solutions. In the case of the resin, it involved weighing 8 grams of the slowly flowing material into a 0.03-liter (1-ounce) wide-mouth bottle on a Torsion pharmaceutical balance to an accuracy of 2 mg., carefully pipetting 10 ml. of solvent, closing the bottle with a selected oversize cork, slowly forcing the cork well into the neck, tumbling the bottle overnight at 15 r. p. m. at 25° C., and backtaring after solution. Transfer was made as rapidly as possible—i. e., in about 5 seconds—to a precision-grade Gardner-Holdt viscosity tube, which was quickly closed with an oversize cork. Repeated comparisons with standard tubes were made at 25° ± 0.2° C.

TABLE I. PHYSICAL PROPERTIES OF HYDROCARBONS USED[a]

Hydrocarbon	Boiling Point ° C.	n_D^{20} [b]	Cottrell Spread ° C.	Estimated Purity %
n-Pentane	36.0 (36.08)	1.3580 (1.3577)	None	99+
Isopentane	28.0 (27.95)	1.3541 (1.3539)	None	99+
n-Hexane	68.6 (68.74)	1.3751 (1.3750)	None	99+
2-Methylpentane	60.2 (60.27)	1.3715 (1.3716)	None	99+
2,3-Dimethylbutane	58.0 (58.00)	1.3749 (1.3750)	None	99+
Neohexane[c]	49.7 (49.73)	1.3689 (1.3689)	None	99+
n-Heptane	98.6 (98.42)	1.3876 (1.3876)	None	99+
2,2-Dimethylpentane	79.1 (79.21)	1.3822 (1.3824)	None	99+
2,3-Dimethylpentane	89.8 (89.8)	1.3920 (1.3920)	None	99+
2,4-Dimethylpentane	80.5 (80.70)	1.3820 (1.3820)	None	99+
Triptane[d]	80.8 (80.88)	1.3894 (1.3895)	None	99+
n-Octane	125.6 (125.63)	1.3980 (1.3976)	None	99+
Isooctane[e]	99.4 (99.23)	1.3918 (1.3916)	None	99+
n-Nonane	150.85 (150.74)	1.4070 (1.4056)	0.05	"90+"
n-Decane	174.2 (174.04)	1.4146 (1.4120)	0.1	"90+"
Methylcyclohexane	100.9	1.4253	0.2	97
Ethylcyclopentane	103.4	1.4197	None	99.5

[a] Values in parentheses assembled by Francis (*1*).
[b] Determined in Pulfrich refractometer.
[c] 2,2-Dimethylbutane.
[d] 2,2,3-Trimethylbutane.
[e] 2,2,4-Trimethylpentane.

TABLE II. ABSOLUTE VISCOSITIES AT 25° C.
(Beckosol No. 19 solids, 8.0 grams in 10.0 ml. of hydrocarbon)

	Gardner-Holdt	Centipoises
n-Pentane	C	85
Isopentane	E + 1/5	128
n-Hexane	E − 1/5, E − 1/5	120, 120
2-Methylpentane	F − 1/3, F − 1/4	135, 136
2,3-Dimethylbutane	G, G	165, 165
Neohexane	J, J	250,250
n-Heptane	F + 1/5, F + 1/5	145, 145
2,2-Dimethylpentane	J − 2/5	240
2,3-Dimethylpentane	G + 1/5	172
2,4-Dimethylpentane	H	200
Triptane	L/M	310
n-Octane	H − 1/5	193
Isooctane	L − 1/4, L − 1/5	294, 295
n-Nonane	J − 2/5	240
n-Decane	L + 1/8	302

To obtain reproducible results, the resin should not change its viscosity rapidly in storage. The resin selected, Beckosol No. 19 solids, is of the drying type, of 27-gallon oil length. It contains no drier, however, and its behavior at room temperature in bulk is such that no skin has formed in the can after more than a year. That its viscosity changes extremely slowly upon standing is shown by the fact that check solutions in the four hexanes made 10 days apart, and in *n*-heptane made 3 weeks apart, gave identical viscosities. Indeed, its solutions made a year ago in identical samples of neohexane, *n*-heptane, and triptane had viscosities within 2 per cent of the values in Table II (*3*). Further, its solution viscosities at the concentration selected for the present series of hydrocarbons are covered by the standard tubes of the Gardner-Holdt "varnish series".

Table III lists absolute and relative viscosities of solutions of high polymers in pairs of hexanes and heptanes. Good agreement between relative viscosities of the milled crepe in the two normal paraffins is notable together with the fact that in each case the branched-paraffin solution has a higher relative viscosity than the *n*-paraffin solution.

Estimation of Viscosity

Within the present series of resin solutions, it is possible to estimate viscosity by an arbitrary method, based on structural formulas written in the plane of the paper. A somewhat similar method of correlating physical properties of paraffin hydrocarbons has recently been employed with interesting results (*2*).

A plot of log solution viscosity against solvent boiling point is linear in the case of the normal paraffins (Figure 1). Average deviation is ±3 per cent. Thus the viscosity of an *n*-butane solution of this resin would doubtless be very close to 63 centipoises at 25° C.

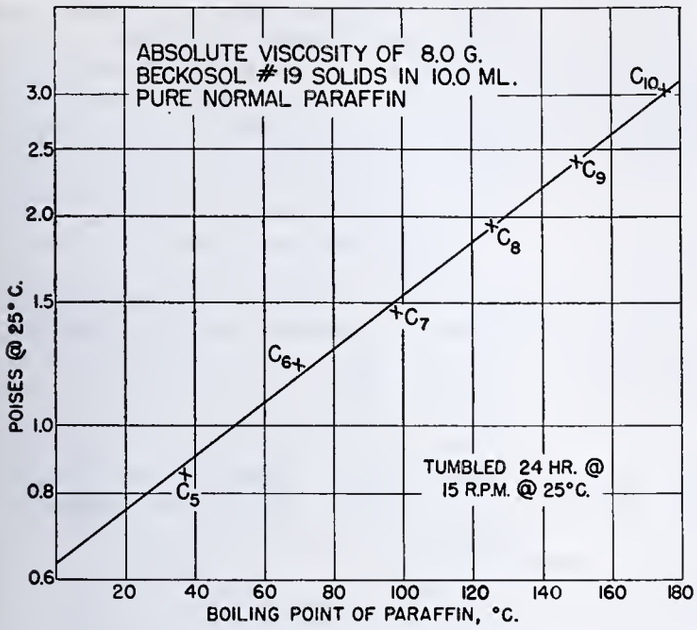


FIGURE 1

If it is now assumed that viscosities of branched-chain paraffin solutions are related similarly to boiling point, for the same degree of branching, an increment can be computed for the effect of branching. For example, isopentane can be considered as the result of adding a methyl branch to butane. The resulting hydrocarbon boils 28.5° C. above butane, and shows a resin solution viscosity of 128 centipoises. It follows, then, that if this branched compound boiled at the boiling point of its parent chain—viz., 28.5° lower—it would show a solution viscosity of 100 centipoises. The estimate is made graphically from Figure 1, as follows: Starting point on the curve is a measured viscosity value (128 centipoises). The curve is followed downward for a difference in boiling points between the parent chain and its branched homolog (28.5°), and the "corrected" viscosity read. Thus, according to the arbitrary assumption, the effect of the degree of branching which makes isopentane out of butane is to increase solution viscosity by 37 centipoises (Table IV).

Placement of a second branch at the adjoining chain carbon, as in 2,3-dimethylbutane, does not increase corrected viscosity. Apparently, once the additional resistance to flow encountered by adding one branch has been overcome, no further resistance is met on adding a second branch to the adjoining chain carbon. This effect is estimated by following the curve downward from its 165-centipoise point for 58.5°, to read the corrected viscosity of 98 centipoises, 2 less than that of isopentane.

When the two branches are separated by one carbon atom, as in 2,4-dimethylpentane, each contributes two thirds the effect it would if alone on the butane chain. Combined effect, of 49 centipoises, is found by following the curve downward from 200 centipoises for 44.5° (the difference between the boiling points of 2,4-dimethylpentane and *n*-pentane) to read the corrected viscosity of 134 centipoises.

The corrected viscosity of the neohexane solution is similarly found to be 159 centipoises, and the branching effect of two methyls on the 2-carbon of butane to be 96 centipoises. Measured viscosities of the other branched paraffin solutions should now be predictable. Table V compares measured and estimated values.

TABLE III. VISCOSITIES AT 25° C.

	Milled Crepe 3.0 Grams in 18 Cc.		Milled Butyl 3.0 Grams in 15 Cc.		Milled Buna S 3.0 Grams in 21 Cc.	
	Absolute *Poises*	Relative	Absolute *Poises*	Relative	Absolute *Poises*	Relative
n-Hexane	20.1	6,700	19.3	6,430	15.3	5,100
Neohexane	35.2	10,350	46.3	13,600	..[a]	
n-Heptane	26.1	6,700	28.8	7,390	20.1	5,140
Triptane	44.6	8,260	63.4	11,750	33.9	6,280

[a] No solution when tumbled at 15 r. p. m. at 25° C.

Triptane may be considered the result of adding a branch to neohexane, at the 3-carbon of the butane chain. In the case of 2,3-dimethylbutane, addition of a second branch at the 3-carbon caused a decrease of 2 centipoises from the corrected viscosity of isopentane. Thus, since two branches at the 2-carbon of butane add 96 centipoises, or 2.5 times 37 centipoises, it is reasonable to expect that the 3-carbon branch of triptane will cause a decrease of 2.5 × 2 centipoises from the corrected viscosity of neohexane, yielding a value of 154 centipoises for the corrected viscosity of triptane.

Increasing Chain Length

Assuming that the contour length of pentane is in the neighborhood of four thirds the contour length of butane, the placement of a methyl branch at the 2-carbon of pentane should produce roughly three fourths the effect it does in the case of butane. On this basis, prediction of the 2-methylpentane solution viscosity is only 4.1 per cent high, as shown in Table V; 2,3-dimethylpentane is 4.6 per cent high; and 2,2-dimethylpentane 3.8 per cent low. It is interesting to note that the absolute solution viscosity of 2,2-dimethylpentane is lower than that of neohexane, although the latter boils 30° C. lower.

In Table IV it is seen that the corrected viscosity contribution of each branch in 2,4-dimethylpentane is equal to two thirds of its value when taken alone as the only branch on the butane chain. The combined branching effect in isooctane may thus be expected to amount to two thirds of 96 (the 2,2-carbon branches) plus 37 (the 4-carbon branch), or 88 centipoises.

Over-all precision involved in making the solutions, transferring to viscosity tubes, and measuring viscosities, is seen from Figure 1 to be about 3 per cent, compared with an aver-

TABLE IV. "CORRECTED" VISCOSITIES AND EFFECT OF BRANCHING

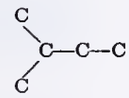
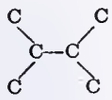
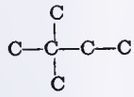
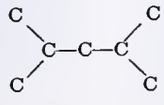
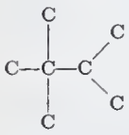
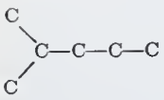
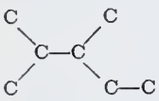
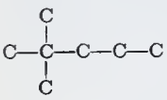
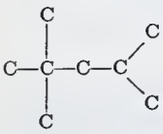
Solvent	Measured Solution Viscosity *Cp.*	Solvent Boiling Point ° *C.*	Temperature Correction ° *C.*	Corrected Viscosity *Cp.*	Net Branching Effect *Cp.*
	128	28.0	28.5	100	37
	165	58.0	58.5	98	35
	250	49.7	50.2	159	96
	200	80.5	44.5	134	49 $\left(=\frac{37+37}{1.5}\right)$

Table V. Estimated Viscosities

Solvent	Net Branching Effect	Corrected Viscosity[a]	Solvent Boiling Point	Temperature Correction	Solution Viscosity[b] Estimated	Solution Viscosity[b] Actual
	Cp.	*Cp.*	° *C.*	° *C.*	*Centipoises*	
	96–5	154	80.9	81.4	316	310
	$^3/_4 \times 37$	113	60.3	24.3	141	135, 136
	$^3/_4 \times 35$	111	89.8	53.8	180	172
	$^3/_4 \times 96$	157	79.2	43.2	231	240
	$\frac{96 + 37}{1.5}$	173	99.2	63.2	300	294, 295

[a] Corrected to boiling point of parent straight chain, which is butane for triptane, pentane for other heptanes, etc.
[b] Average deviation between actual and estimated, 3.3%.

age deviation of 3.3 per cent between measured and estimated values. Following customary practice in correlating properties of hydrocarbons, the method was fitted to existing data on available hydrocarbons. The present data do not provide for estimates in such structures as 3-methylpentane, 3-ethylpentane, 3-methylhexane, 3,3-dimethylpentane, or 2,3,4-trimethylpentane. These paraffins, however, are less likely to be present as major components of postwar commercial solvent naphthas than some of the products studied.

Trial Analytical Method

At the present writing, there exists no wholly satisfactory means of determining the relative proportions of normal paraffins, branched paraffins, and naphthenes in close-cut naphtha fractions. The large spreads in resin solution viscosity between normal and highly branched paraffins offer a basis for such a method. As shown in Table II, isooctane has a 103 per cent higher solution viscosity than *n*-heptane, whereas its density is only 1.5 per cent higher, and its refractive index only 1 per cent higher than the corresponding values for *n*-heptane. Indeed, refractive index is customarily used to estimate naphthenic content, since within a narrow boiling range the spread between paraffins averages less than one fifth that between paraffins and the naphthene average (*1*, *4*).

A number of potential errors preclude basing an accurate analytical procedure upon solution viscosities obtained in the present series, although it is possible to make a rapid semiquantitative estimate of hydrocarbons present. Beckosol No. 19 solids does not behave ideally (Table VI). It contains a terpene derivative, which may explain its high viscosity in methylcyclohexane as compared with its solutions in ethylcyclopentane, *n*-heptane, or the 50/50 mixture with *n*-heptane. (Solution viscosity measurements when the solvents boil around 100° C. show a precision of 2 per cent or less, since very little evaporation occurs during transfer to viscosity tubes, and the same standard tubes are used for several comparisons.) Further, it is seen from Figure 2 that when naphthenes are present the log solution viscosity–solvent composition curve is not linear, as in the case of mixed paraffins (Table VI). Moreover, the curve must be smoothed to begin at 152 centipoises, an ideal viscosity for the 50/50 methylcyclohexane–*n*-heptane blend, rather than at the actual 140-centipoise point. Thus when less than about 50 per cent of isooctane is present, the expected error is relatively large.

A test sample containing 28.55 per cent *n*-heptane, 28.55 per cent isooctane, and 42.9 per cent methylcyclohexane showed a solution viscosity of 172 centipoises and a refractive index of 1.4055. Taking 1.3900 as the refractive index of the paraffins present, the methylcyclohexane content would be estimated at 44 per cent. Then, from Figure 2, the isooctane content is read as 33 per cent. (If the isooctane content could be determined accurately, its contribution to refractive index could be subtracted from 1.4055, and the remainder partitioned algebraically between *n*-heptane and methylcyclohexane. Meanwhile, the round figure 1.3900 is taken as an average.)

It is hoped that further work will disclose a stable resin, or other polymer, which exhibits a minimum differential in the viscosity of its solutions between normal paraffins and naphthenes of the commonly encountered

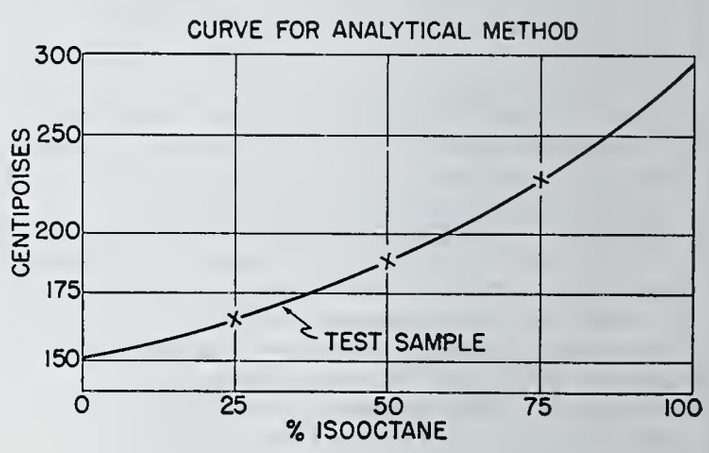


Figure 2

Table VI. Solution Viscosities and Refractive Indices

Solvent	Solution Viscosity	n_D^{20}
	Cp.	
n-Heptane	145, 145	1.3876
Methylcylohexane	160, 155	1.4253
Ethylcyclopentane	130, 128	1.4197
Isooctane	294, 295	1.3918
50/50 methylcyclohexane/*n*-heptane[a]	140, 140	1.4067
75% A/25% isooctane	165	1.4031
67% A/33% isooctane	172	1.4021
50% A/50% isooctane	188	1.3995
25% A/75% isooctane	227	1.3958
50/50 *n*-heptane/isooctane	202	1.3900
50/50 methylyclohexane/isooctane	191	1.4090
33/33/33 *n*-heptane/ethylcyclopentane/isooctane	165	1.4005
Test sample	172	1.4055

[a] Mixture A.

types, but which when dissolved at a suitable concentration gives a maximum spread between normal and branched paraffins. Tabulated data covering the necessary number of 10° C.-wide fractions can then be taken once, and the viscosity of an unknown sample determined at a later date concurrently with a control determination on a selected normal paraffin, to correct for resin or polymer aging.

Acknowledgment

Opportunity is taken to thank M. Lapeyrouse of the Esso Laboratories, Research Division, for helpful criticism in connection with this work. The authors are also indebted to M. R. Fenske, of the Pennsylvania State College, for supplying the majority of hydrocarbons studied.

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N-Methylaniline Point of Viscous Petroleum Oils

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During the past twenty years, aniline point (*1*, *9*) has become accepted as a reliable quick estimate of the proximate composition of the lighter straight-run petroleum products. It has been widely adopted as a measure of solvency of petroleum distillates for various solutes, and has acquired its popularity largely because among such tests it alone requires no temperature control. For highly aromatic solvent naphthas, it has more recently been modified to "mixed aniline point" (*5*, *6*, *8*), wherein the aromatic naphtha is first diluted with an equal volume of a 60° C. aniline point naphtha of 43°/44° API gravity. For testing lubricating oils of relatively high aniline point—i. e., above 80° C.—a second modification is now suggested, wherein aniline is replaced with *N*-methylaniline. Preliminary results indicate that the method may also serve in testing other petroleum products. The following simple relation exists between aniline point and *N*-methylaniline point of petroleum lubricating oils:

No. of Oils Tested	Range: Aniline points ° C.	Range: *N*-Methylaniline points ° C.	Temperature Difference ° C.
15	80–130	3–53	77 ± 1

At present, an important use of this type of test is in estimating the relative tendencies of these oils to attack rubberlike materials. Following the development of several synthetic elastomers which are not easily soluble in petroleum oils, an increasing proportion of flexible transfer lines and storage equipment has been fabricated from these materials, and correlations have been drawn between the aniline point of petroleum oils and the logarithm of the per cent increase in volume of rubberlike materials (*2*, *4*, *7*).

The proposed test method was developed because laboratory determinations of aniline point become increasingly difficult and hazardous as one passes from the lighter petroleum fractions to such heavy and highly paraffinic stocks as aviation lubricating oils. At 120° C. (248° F.) a hot oil or hot air bath, or both, is required to obtain an accurate reading. Furthermore, experience shows that the vapor pressure of aniline at this temperature is sufficient to volatilize enough from the stirred mixture to cause a progressive change in a series of readings. Moreover, the concentration of aniline vapor in the vicinity of a short test tube may be deemed objectionable by sensitive operators.

Aniline Point of Diluted Oil

In an attempt to lower the equivalent aniline point of lubricating oils, a typical 120-viscosity aviation oil was diluted with an equal volume of toluene, and the aniline point of this mixture determined. It was hoped that such dilution would drop the temperature to the half-way point, much as "mixed aniline point" raises the reading for aromatic naphthas. (Here, the straight aniline point of toluene is assumed to be −40° C., −40° F., based upon its "mixed aniline point"

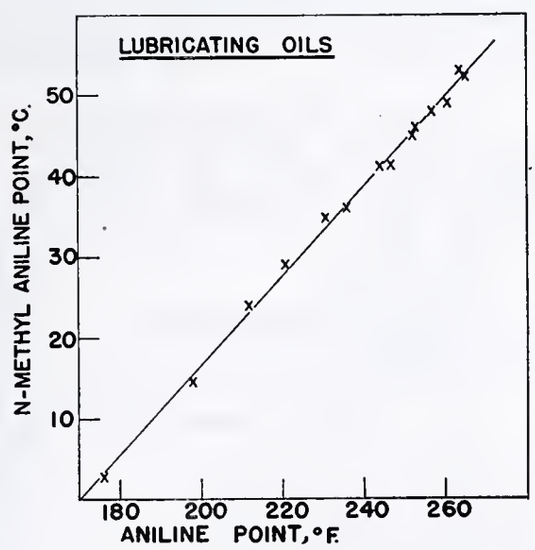

Figure 1

of 10.0° C.) Unfortunately, however, instead of lowering the aniline point of the aviation oil (255° F.) to the half-way point (107.5° F.)—i. e., by 147.5° F.—the drop amounted to only 85° F., from 255° to 170°. When the oil was diluted with two volumes of commercial 10° xylene, to make 10 ml. of mixture, the critical solution temperature with 10 ml. of aniline became 140° F., much more than one third the way from −40° (assumed for xylene, from its mixed aniline point of 10° C.) up to 255°. Thus no simple correlation between aniline point and a blended aniline point appeared likely to exist when dealing with such dissimilar materials as low-boiling aromatics and viscous lubricating oils. When *N*-

Table I. Inspections of *N*-Methylaniline Used

Specific gravity at 25° C.	0.981	50% distilled, ° C.	197.0
n_D 20° C.	1.5682	90% distilled, ° C.	197.0
Initial boiling point, ° C.	195.1	95% distilled, ° C.	197.2
5% distilled, ° C.	196.4	Dry point, ° C.	198.4
10% distilled, ° C.	196.7		

ethylaniline was substituted for aniline and no diluent was present, complete miscibility occurred with the aviation oil at room temperature.

N-Methylaniline Point

N-Methylaniline was accordingly tried. The ideal temperature range of a satisfactory substitute test for the aniline point of lubricating oils would (1) be exactly as wide as the corresponding temperature range of their aniline points—i. e., about 50° C. (90° F.)—and (2) for practical convenience, begin just above melting ice temperature for the lowest values ordinarily encountered, and not extend above 60° C. (140° F.), easily obtainable with running hot water. Table II shows how ideally *N*-methylaniline meets these requirements. With the wide variety of lube oils tested, it drops the aniline point by 77° ± 1° C., or 139° ± 2° F. Beginning with 3° C. for an SAE 10 coastal (naphthenic) oil, *N*-methylaniline values parallel aniline values upward to 53° C., for a treated Pennsylvania 120-viscosity aviation lube oil, as indicated in the last column of Table II. The correlation between aniline point and *N*-methylaniline point is shown in Figure 1, and the relationship between these values and swelling tendency is indicated in Figure 2.

Table II. Aniline Points and *N*-Methylaniline Points

Oil	Viscosity at 210° F.	Viscosity Index	Aniline Point[a] ° F.	Aniline Point[a] ° C.	*N*-Methylaniline Points: Operator 1 ° C.	Operator 2 ° C.	Operator 3 ° C.	Av. ° C.	Av. deviation ° C.	Av. deviation ° F.	Difference between Aniline and *N*-Methylaniline Points (Operator 1) ° C.
SAE 10 coastal	40	30	176	80.0	2.9	2.6	2.8	2.8	0.1	0.2	77.1
SAE 40 coastal	69	35	198	92.2	14.6	14.3	14.0	14.3	0.3	0.5	77.6
Penn. 180 neutral	45	102	212	100.0	24.0	23.2	23.2	23.5	0.4	0.7	76.0
Nujol	47	80	221	105.0	29.0	29.4	29.1	29.2	0.2	0.4	76.0
SAE 10 extracted mid-continent	44	113	231	110.6	34.8	34.7	34.3	34.6	0.2	0.4	75.8
Coastal bright stock	190	65	236	113.4	36.0	36.5	36.7	36.4	0.3	0.5	77.4
SAE 50 mid-continent	100	96	244	117.8	41.2	40.8	41.0	41.0	0.1	0.2	76.6
Calif. aviation 120	120	87	247	119.4	41.5	41.0	40.8	41.1	0.3	0.5	77.9
Penn. bright stock	150	98	252	122.1	44.9	45.0	45.0	45.0	0.1	0.1	77.2
Extracted mid-continent av. 100	100	103	253	122.8	46.0	46.8	45.7	46.2	0.4	0.8	76.8
Extracted mid-continent av. 120	120	103	257	125.0	48.0	47.3	47.7	47.7	0.2	0.4	77.0
Treated Penn. aviation 120	123	104	257	125.0	48.0	47.6	47.7	47.8	0.2	0.3	77.0
Extracted mid-continent 150 bright stock	145	102	261	127.2	49.0	49.0	49.0	49.0	0.0	0.0	78.2
160 viscosity extracted bright stock	160	95	265	129.4	52.3	52.0	52.7	52.3	0.2	0.4	77.1
Treated Penn. aviation 120	120	105	264	128.9	53.0	53.0	53.0	53.0	0.0	0.0	75.9

[a] Taken by operator 1, in A. S. T. M. steam emulsion tubes; believed equal to strict A. S. T. M. aniline point (*1*).

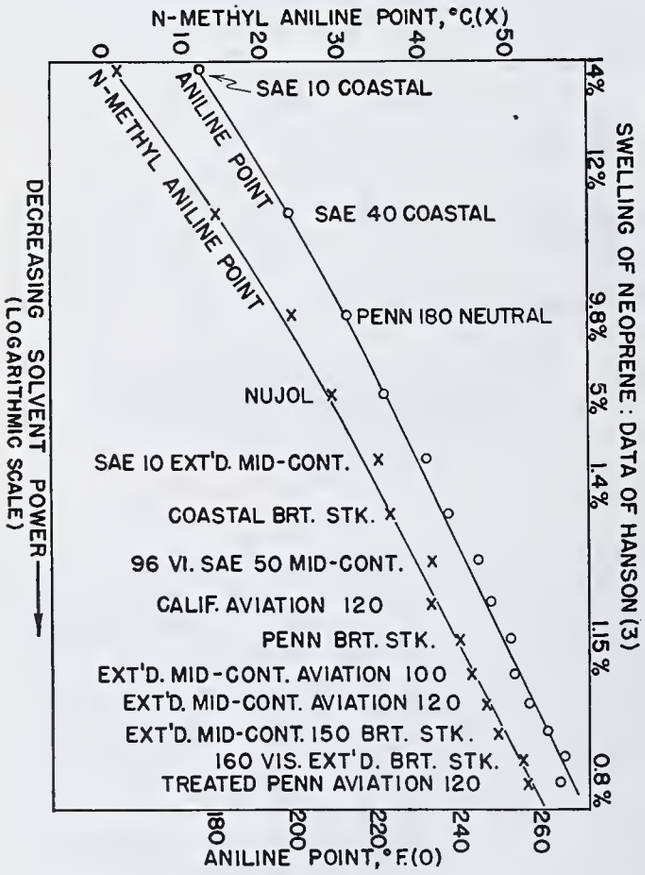

Figure 2

Table III. Possible Errors

Effect of Incorrect Measurement

Mixture	Critical Solution Temperature ° C.
9 ml. of SAE 10 coastal, 11 ml. of *N*-methylaniline	4.1
10 ml. of SAE 10 coastal, 10 ml. of *N*-methylaniline	2.9
11 ml. of SAE 10 coastal, 9 ml. of *N*-methylaniline	1.6
9 ml. Pennsylvania bright stock, 11 ml. of *N*-methylaniline	46.8
10 ml. Pennsylvania bright stock, 10 ml. of *N*-methylaniline	44.9
11 ml. Pennsylvania bright stock, 9 ml. of *N*-methylaniline	43.5

Effect of Redistillation

	Six Weeks Old Sample ° C.	Freshly Distilled ° C.
Equal volumes of SAE 10 coastal and *N*-methylaniline	2.9	2.6
Equal volumes of Pennsylvania bright stock and *N*-methylaniline	44.9	44.6

Effect of Added Water

	° C.
N-methylaniline point of SAE 10 coastal oil	2.9
Same, plus 0.04 ml. of water	3.0
N-methylaniline point of Pennsylvania bright stock	44.9
Same, plus 0.04 ml. of water	45.4

The *N*-methylaniline employed was the Eastman grade labeled "free of aniline and dimethylaniline". It was six weeks old when used. Pertinent inspections are shown in Table I. A double redistillation, discarding light and heavy ends, caused a negligible change in the *N*-methylaniline points of the light coastal oil and a Pennsylvania bright stock (Table III).

Presumably, like aniline, *N*-methylaniline should be handled in the laboratory as a toxic reagent, although accord-

ing to one authority (*3*) "introduction of an alkyl group such as methyl (CH_3) . . . makes (*N*-methylaniline) less poisonous than aniline, for dimethylaniline is less poisonous than aniline." Like aniline, *N*-methylaniline is an established intermediate in the dyestuff industry.

Performance of the Test

The current A. S. T. M. method for aniline point (A. S. T. M. D611-41T) calls for pipetting the aniline, and pipetting or weighing the sample to be tested. Since the room-temperature pipetting of a 120-viscosity at 210° or heavier aviation oil is practically impossible, the sample and test tube must each be weighed, a time-consuming process. Hence resort was made to the utilization of A. S. T. M. steam emulsion tubes (A. S. T. M. D157-36) of Pyrex, 200 mm. long and of 23-mm. bore (8 × 1 inch test tubes), graduated in milliliters beginning at 10 ml. The tubes had been previously calibrated by the Standard Inspection Laboratory, Standard Oil Development Company, Bayonne, N. J. Correct placement of the bottom line of the graduated range, at 10 ml., is as important to the *N*-methylaniline point test as are the other graduations. Selected tubes were rechecked for the accuracy of this marking against a 10.0-ml. pipet, and were all found correct. The reagent, being heavier than lubricating oils, is poured into the tube first. The bottom of its meniscus is tangential to the marking, which encircles the tube. Oil to be tested is poured atop the reagent, again with the meniscus tangential to the top of the 20-ml. marking.

Interfacial tension between glass and *N*-methylaniline is apparently much lower than in the case of aniline, since the former flows smoothly down the wall of the tube, leaving fewer and smaller droplets above the body of the liquid.

The precision of the test is seen from Table II to be ±0.2° C., or ±0.4° F. Table III shows that the effect of cumulative 10 per cent errors in measuring the components—fivefold those indicated as the average of the three operators—is about 1.5° C. over the range of oils tested; and the presence of water raises the *N*-methylaniline point, as it does the aniline point.

Conclusions

N-Methylaniline point has practical advantages as a qualitative test instead of aniline point for the proximate composition of petroleum oils. Aniline points may be obtained from *N*-methylaniline points by adding 77° C. or 139° F. A. S. T. M. steam emulsion tubes offer a convenient and precise means of performing the test.

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Determination of Free Gossypol in Cottonseed Meal

A Colorimetric Method

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IT HAS been shown by Lyman, Holland, and Hale (*4*) that cottonseed meal can be processed in such a manner that it no longer has any toxic qualities whatsoever, and that the procedure is a practical one from the standpoint of oil mill operation. It has also been shown that the free gossypol content of cottonseed meal is a reliable index which will determine whether any given sample of meal will prove toxic in animal feeding tests.

In the authors' experience, accurate results for the determination of free gossypol by the precipitation of the dianiline compound from extracts of cottonseed meal have been obtained only with considerable difficulty. In attempting to follow the procedure of Halverson and Smith (*2*, *5*) duplicate determinations often gave satisfactory checks but the precipitates were not always sufficiently pure to give the accuracy desired, and precipitates which had the color of dianiline gossypol were never obtained.

This paper reports a quantitative method for the determination of gossypol, based on the change in color which occurs when aniline reacts with gossypol in an organic solvent.

Experimental

The absorption spectra data on which the method is based were obtained with a Cenco Sheard spectrophotelometer. For purposes of routine analysis any good photoelectric colorimeter with a filter transmitting light of 440-millimicron wave length is satisfactory. The procedure used for the isolation of gossypol to be used as a standard was the same as that given by Campbell, Morris, and Adams (*1*).

Figure 1 shows the comparison of the absorption of light by pure gossypol and by dianiline gossypol (formed by the addition of aniline to the solvent), both measured against a reference cell containing pure solvent. Dianiline gossypol exhibits a maximum of absorption at 440 millimicrons while pure gossypol absorbs very little light at this wave length.

By measuring the absorption of a solution of gossypol plus aniline (freshly distilled, water-white) against a reference cell containing the same amount of gossypol but no aniline, the change in absorption due to the reaction with aniline can be measured. This procedure makes it possible to apply the measurement to solutions containing other colored materials besides gossypol, provided that gossypol is the only substance present which reacts with aniline to produce a color change.

Figure 2 shows this type of measurement applied to solutions of gossypol at two concentrations and to a diluted ether extract of cottonseed meal. The close similarity in the shapes of the absorption curves suggests that the change in absorp-

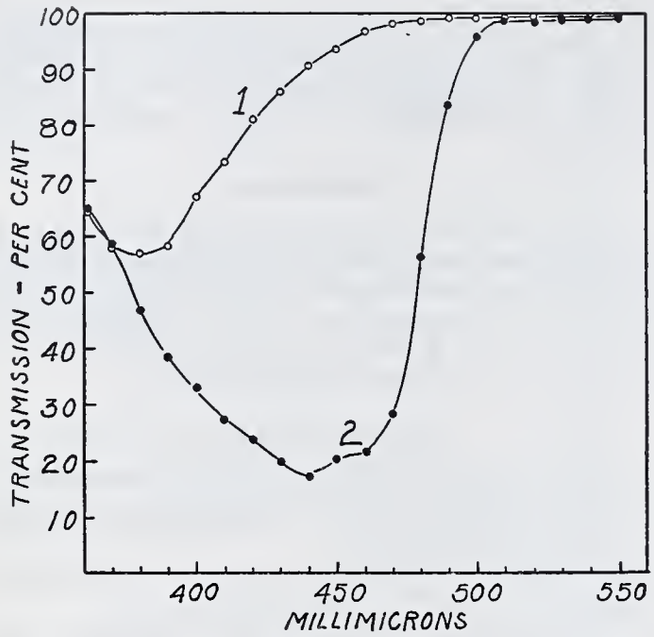

FIGURE 1. ABSORPTION SPECTRA OF GOSSYPOL AND DIANILINE GOSSYPOL

Cell thickness, 5 cm.

1. 0.05 mg. of gossypol in 25 ml. of ethyl ether–butanol mixture
2. 0.05 mg. of gossypol, 2 ml. of aniline-ether-butanol mixture to make 25 ml.

The portion of the absorption spectra between 550 and 750 millimicrons wave length was omitted because at the concentrations used there was practically no absorption in this region.

TABLE I. TIME REQUIRED FOR DEVELOPMENT OF COLOR

(Amount of gossypol, 0.18 mg. in 25 ml. of test solution. Cell thickness, 1 cm.)

Time, Minutes	Log $\frac{I_0}{I_1}$
3	0.393
6	0.432
9	0.456
12	0.469
17	0.475
20	0.481
27	0.480
35	0.481
60	0.481
120	0.480
180	0.480

tion resulting from the addition of aniline to the extract of cottonseed meal is essentially due to the gossypol present.

However, aniline does react with certain other compounds having a carbonyl group attached to an aromatic nucleus. By way of comparison, the change in absorption spectra which occurs when aniline reacts with two of these compounds is given in Figure 3.

It appears that if there are other substances besides gossypol in ether extracts of cottonseed meal, which give color with aniline, these substances must be closely related to gossypol in chemical structure.

Figure 4 shows that the extinction coefficient of the color change is a straight-line function of the concentration of gossypol when the gossypol concentration lies between 0.05 and 0.30 mg. per 25 ml. of solution.

It was found that the time necessary for the development of maximum color varies with the amount of aniline used. Maximum color was always developed in 20 minutes when 2 ml. of aniline in a total volume of 25 ml. were used. After the color was developed it was stable for 3 hours at least (Table I).

TABLE II. RECOVERY TESTS

Gossypol in Extract	Gossypol Added	Gossypol Found	Recovery of Added Gossypol
Mg.	*Mg.*	*Mg.*	%
0.070	....	0.070	...
0.070	0.0235	0.094	102
0.070	0.047	0.118	102

Extraction of Free Gossypol from Cottonseed Meal

The extraction of 2-gram samples wrapped in filter paper and placed in Butt extractor tubes gave results equally as consistent as the extraction of the same or larger quantities of material in Soxhlet extractors. When large numbers of samples are to be run, the use of the simpler apparatus is of considerable advantage. A search was made for a solvent which would extract the free gossypol more rapidly than ethyl ether but none was found. Because the authors' data concerning the following points are in agreement with the conclusions of Halverson and Smith (*3*, *5*) they are omitted here:

Anhydrous ether extracts very little free gossypol from cottonseed meal.

Moistening the sample and adding small amounts of water to the ether increase the amount of gossypol extracted.

The use of ether containing 2.3 to 2.5 per cent of alcohol in addition to small amounts of water further increases the amount of gossypol extracted.

Even after extraction periods as long as 3 to 4 days, further extraction yields additional small quantities of gossypol. There seems to be no sharp boundary between free and bound gossypol.

Detailed Procedure

A 2-gram sample of cottonseed meal ground to pass a 40-mesh sieve is wrapped in a 125-mm. filter paper (S. & S. No. 597 or equivalent grade) and rewrapped in a second filter paper, the top being left open like a thimble. The sample is then placed in a Butt extractor tube and a piece of absorbent cotton is introduced into the top of the thimble to distribute the dropping solvent. A 250-ml. Erlenmeyer flask serves as an extraction vessel. Extraction with 60 ml. of peroxide-free ethyl ether containing 2.3

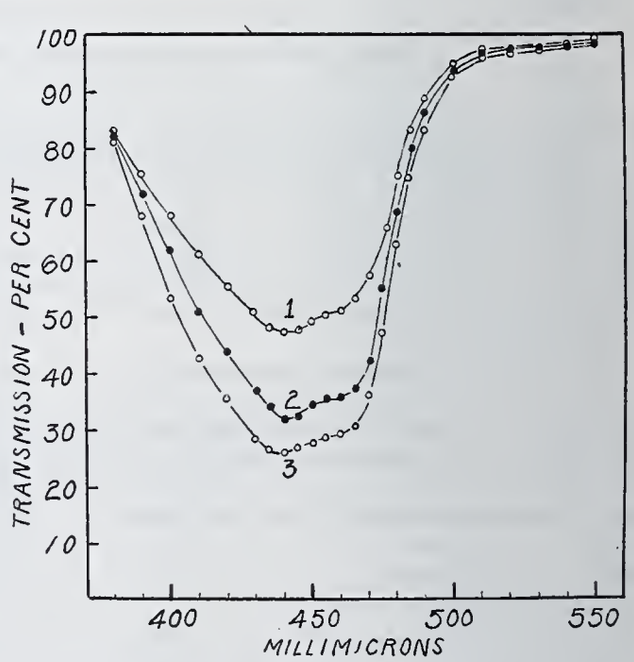

FIGURE 2. CHANGE IN ABSORPTION SPECTRA ON ADDITION OF ANILINE

Solvent, *n*-butanol. Amount of aniline, 2 ml. in total volume of 25 ml. Cell thickness, 5 cm.

1. Pure gossypol, 0.02 mg.
2. Diluted ethyl ether extract of cottonseed meal
3. Pure gossypol, 0.04 mg.

to 2.5 per cent alcohol and 1 to 1.2 per cent water (by weight) is continued for 72 hours. The solvent should fall from the condenser on the center of the thimble at the rate of at least 150 drops per minute.

At the end of the extraction period about 5 ml. of *n*-butanol are added to the flask and the ether is removed at reduced pressure while the flask is rotated in a pan of warm water. The residue is transferred to a 25-ml. volumetric flask with the aid of a butanol wash bottle and a small funnel. *n*-Butanol is used to dilute to the mark. Two aliquots (usually 2 to 5 ml., depending on the gossypol content) are transferred to two other 25-ml. volumetric flasks; 2 ml. of freshly distilled aniline are added to one of these and both flasks are diluted to the mark with *n*-butanol. After about 20 minutes the solutions are transferred to the cells of a photoelectric colorimeter or spectrophotometer and relative measurements of the light transmission of the two solutions are made at 440-millimicron wave length. The amount of gossypol is directly proportional to log $\frac{I_0}{I_1}$ where I_0 is the intensity of the light transmitted without aniline and I_1 is the intensity of the light transmitted by the solution containing aniline. A standard curve is prepared using values obtained with pure gossypol. Any good photoelectric colorimeter or spectrophotometer may be used, but it is necessary to prepare a standard curve for each instrument. The aliquots used for the determination should contain from 0.05 to 0.30 mg. of gossypol when spectrophotometer cells of about 1 cm. thickness are used.

Recovery tests were carried out by analyzing ether extracts of cottonseed meal both before and after the addition of known amounts of pure gossypol. The results given in Table II indicate that the presence of other colored compounds in ether extracts of cottonseed meal does not interfere with the accuracy of the determination.

Table III. Duplicate Determinations of Free Gossypol in Cottonseed Meal

Sample No.	Free Gossypol Content Determination 1	Determination 2
	%	%
83	0.045	0.046
90	0.098	0.100
100	0.021	0.022
110	0.118	0.115
111	0.079	0.080
5	0.020	0.022
5	0.022	0.021

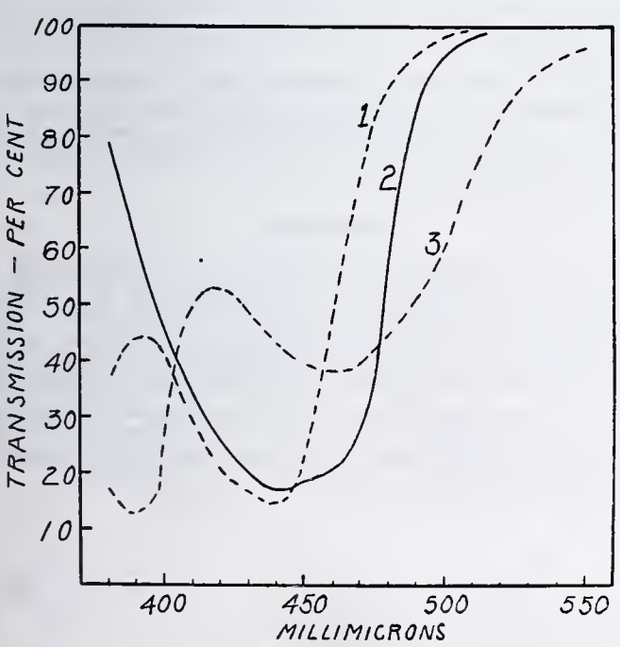

Figure 3. Change in Absorption Spectra Due to Reaction with Aniline

Cell thickness, 5 cm.

1. Dibromosalicylaldehyde, 1.0 mg. in 25 ml.
2. Gossypol, 0.05 mg. in 25 ml.
3. 3,4-Dihydroxybenzaldehyde, approximately 2.4 mg. in 25 ml.

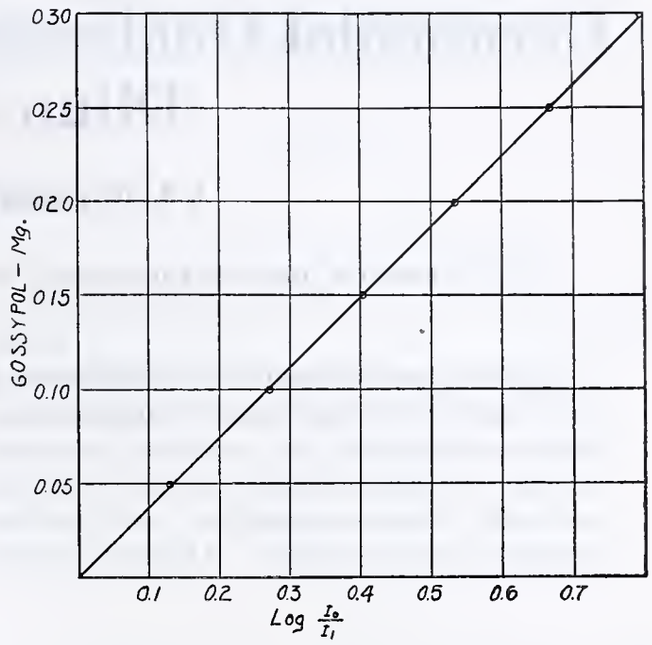

Figure 4. Standard Curve for the Colorimetric Determination of Gossypol

Wave length, 440 millimicrons. Cell thickness, 1 cm.

Table III gives the free gossypol content of a number of samples of cottonseed meal. These data are included in order to illustrate to what extent duplicate analyses may be expected to check each other.

Discussion

The method outlined is easily performed and involves no detailed, difficult procedures. There appears to be no source of error due to other pigments present in cottonseed meal. The method is particularly useful for accurately determining extremely small quantities of free gossypol in cottonseed meal. In such cases the solubility of dianiline gossypol becomes a source of error in the determination by the precipitation method.

The procedure may be modified by moistening the samples with water before extraction and adding water to the ether in the extraction flasks. Higher values are obtained by doing this. Evidence is given in another report (*4*) that the determination of free gossypol according to the procedure given above offers a method of ascertaining when cottonseed meal has been processed in such a manner that it will have no residual toxic qualities whatsoever. When values for the free gossypol content of cottonseed meal are compared, due consideration should be given to the exact conditions of extraction.

Acknowledgment

Since 1942, the program of research on cottonseed products at the Texas Agricultural Experiment Station has been supported in part by a grant-in-aid from the Texas Cotton Research Committee.

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Germicidal Quaternary Ammonium Salts in Dilute Solution

A Colorimetric Assay Method

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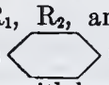
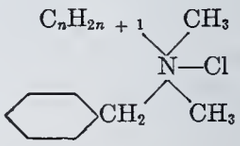
A colorimetric method for the estimation of some germicidal quaternary compounds in dilute solutions is, for practical purposes, specific in that inorganic alkalies and the common primary, secondary, and tertiary amines do not interfere. Therefore an extension of the general method has been suggested for the estimation of alkaloids (cations) with bromothymol blue as dye anion, and for the estimation of some colored sulfonic acids (anions) with quaternary ammonium cations.

THE quaternary ammonium compounds which are now becoming increasingly useful as antiseptics and germicides (*1, 4, 5, 6, 8, 11, 12, 13*) are of such potency that solutions more concentrated than 1 to 500 are seldom used. Quite properly, these compounds are usually standardized on the basis of their phenol coefficient, or actual bactericidal efficiency. Several years ago, however, this laboratory was given the problem of devising a chemical assay method sufficiently simple for field use. Such a method was worked out, and has proved to be of value not merely in the field, but also (with suitable refinements) as a laboratory control test.

The quaternary ammonium salts named below form colored salts with dibromothymolsulfonphthalein (bromothymol blue) and with tetrabromophenolsulfonphthalein (bromophenol blue). These salts are readily extracted from alkaline aqueous solutions by a number of organic solvents, particularly the chlorinated solvents. Bromophenol blue, which the the author prefers to use, is itself insoluble in ethylene dichloride (for example), either in its acid form or as the sodium salt; nor does it form salts extractable from alkaline solution with any of a large number of primary, secondary, and tertiary amines at the author's disposal, or with alkaloids. It seems probable that all cations of the composition $[R_1R_2R_3R_4N]^+$ where R_1, R_2, and R_3 are CH_3 or longer chained alkyls and R_4 is  CH_2, C_4H_9, or longer chained aryl-alkyl, will form salts with bromophenol blue which can be extracted from alkaline solution. It is not claimed that only quaternary amines respond to this test, but it is true that of 50 or 60 nonquaternary amines, all gave negative tests. These negative compounds include such typical amines as ethylamine, dimethylamine, diethanolamine, diethylaminoethanol, aniline, phenylenediamine, and ethylenediamine. It is especially significant that lauryl dimethylamine, which can be considered a possible impurity in alkyl benzyl dimethyl ammonium chloride, gives a negative test.

The method consists of forming the quaternary ammonium-dye salt in carbonate solution, extracting the color with ethylene dichloride, and measuring in a photoelectric colorimeter the intensity of color so extracted. By means of a factor previously derived from a standard solution, the concentration of quaternary ammonium salt in the sample is readily calculated.

To illustrate the method under discussion, there is described below the assay of a commercial tinted alcohol-acetone solution containing 1 to 1000 alkyl benzyl dimethylammonium chloride (referred to as *Z*):



in which C_nH_{2n+1} represents a mixture of alkyl groups ranging from C_8H_{17} to $C_{18}H_{37}$. In spite of the apparently indeterminate constitution of this substance, the average molecular weight, because of rigidly standardized manufacturing conditions, is remarkably constant, being about 357.5. The molecular weight was determined by preparing the ferricyanide salt $R_3Fe(CN)_6$ (R = quaternary ammonium cation), igniting a portion of the salt to Fe_2O_3, and calculating back to the value of R.

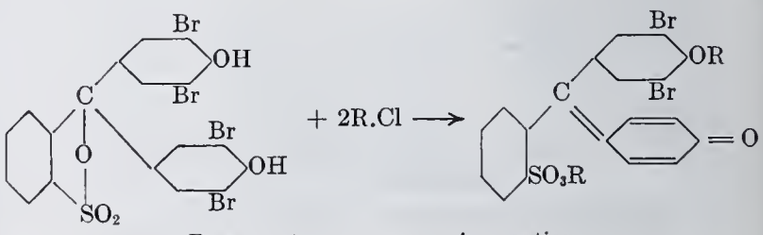
Standard Solution

Titrate a 10 per cent solution of *Z* by the ferricyanide method (*3*). From this standard prepare a working dilution containing 0.1 mg. per ml.

In working with other quaternary salts, a standard solution of the compound in question might be used in place of a standard *Z* solution. The discussion below will apply equally to the new standard, necessitating only the obvious changes resulting from a different molecular weight.

Method

Dilute the 1 to 1000 tinted tincture exactly ten times with distilled water. Take 2 ml. of the dilution (about 0.2 mg. of quaternary salt) in a 125-ml. glass-stoppered Erlenmeyer flask. Add 2 ml. of 10 per cent sodium carbonate solution and 1 ml. of 0.04 per cent aqueous bromophenol blue indicator solution, dilute with about 50 ml. of distilled water, add exactly 10 ml. of ethylene dichloride, stopper the flask, and shake gently for one minute. One gram-molecule of dye reacts with 2 gram-molecules of quaternary salt in a manner analogous to the normal salt formation of the sulfonphthaleins (*2*):



R = quaternary ammonium cation

Table I. Assay of Ammonium-Dye Salt Z

Z in Sample	Instrument Reading
Mg.	
0.05	38
0.10	77
0.20	152
0.30	230
0.40	300
0.50	376

To samples containing more than 0.3 mg. of Z, an extra ml. of indicator solution was added.

That is, 357.5 mg. of Z react with 335 mg. of bromophenol blue, or 1 mg. of Z requires 0.94 mg. of bromophenol blue. Further, a linear relationship holds throughout a desirable range of concentrations, as demonstrated in Table I.

When the layers have separated, aspirate off the aqueous layer, transfer the colored ethylene dichloride layer to a dry test tube, add 0.5 to 1 gram of dry sodium sulfate, and mix by gentle agitation. Decant the clarified colored solution into a Klett-Summerson photocolorimeter tube and read in the instrument, using filter No. 54, or equivalent instrument and filter. In the same way run the prepared standard dilution.

Use of the Calculation Factor

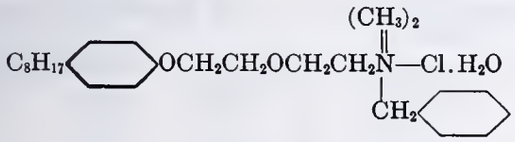
When colored solutions, which follow Beer's law, are read in a photoelectric colorimeter provided with a logarithmic scale, it is true that

$$RS \times K = CS$$

where RS = instrument reading of standard
CS = concentration of standard
K = a constant

In the case of a 1 to 1000 standard solution assayed as described above, the colored standard solution read in the instrument contained the equivalent of 0.2 mg. of original quaternary salt per 10 ml.—i. e., 0.002 per cent. The actual reading on the author's instrument was 152. Then $K = \frac{0.002}{152} = 0.0000131$, or, to put it otherwise, the instrument reading $\times$ 0.00131 = mg. of anhydrous quaternary salt in the 2-ml. dilution taken for analysis. This factor, naturally, is valid for only one particular instrument and filter combination. Once the factor has been determined, it becomes unnecessary to prepare further standard solutions. One simply makes a convenient dilution of the unknown and takes a [illegible] containing about 0.2 mg. of substance for test. The [illegible]entration is then calculated from the instrument reading and K, as indicated. Further, since the formation of the colored salt is a stoichiometric relationship, and since the spectral characteristics of the color have to do only with the dye anion and are independent of the particular quaternary cation in use, it is unnecessary to prepare standard solutions of new quaternary compounds, so long as the molecular weight of the new compound is known. It is only necessary to recalculate K to fit the new molecular weight.

For example, when solutions of *p-tert*-octyl phenyl diethoxy dimethyl benzyl ammonium chloride monohydrate (*11*):



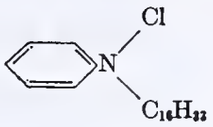
became available, K was recalculated as follows:

$$K_2 \text{ (for new substance)} = K_1(Z) \times \frac{465.5 \text{ (molecular weight of new substance)}}{357.5 \text{ (molecular weight of } Z)}$$

$$K_2 = 0.00131 \times 1.302 = 0.0017$$

Using the new factor, K_2 = 0.00170, an aqueous solution of the new compound labeled 1 to 1000 was assayed and 0.998 gram per liter found. A tinted tincture labeled 1 to 500 assayed 1.997 grams per liter, using the same factor.

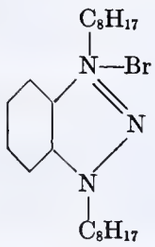
Similarly, for cetyl pyridinium chloride (*12, 13*):



$$K_3 = 0.00131 \times \frac{339.5}{357.5} = 0.00124$$

Using the factor K_3 = 0.00124, a tinted tincture labeled 1 to 4000 assayed 0.220 gram of anhydrous cetyl pyridinium chloride per liter.

Again, for 1,3-di-*n*-octyl benztriazolium bromide (*6*):



K_4 was calculated as $0.00131 \times \frac{424}{357.5} = 0.00155$.

Using the factor K_4 = 0.00155, a solution of the pure substance made up in a concentration of 1 to 1000 assayed 1.020 grams per liter.

Possible Analytical Uses of General Method

Alkyl benzyl dimethyl ammonium chloride (and other quaternary salts, no doubt) forms salts with some sulfonic acid derivatives, the salts being extractable from water solution. For example, sulfanilyl azo acetyl 2R acid (Neoprontosil) is very soluble in water. On addition of the quaternary compound, the resultant salt can be extracted quantitatively with a number of water-immiscible solvents.

Under the specified test conditions (more specifically, in alkaline medium) the dye salts of alkaloids are not extractable. However, these salts become extractable from acid solutions, and useful methods can be developed for the determination of minute amounts of various alkaloids in solution. In fact, such a method has already been found useful for the determination in urine of an alkaloidlike synthetic drug, the ethyl ester of methyl phenyl piperidine carboxylic acid (*7*).

In this laboratory very small amounts of strychnine have been determined in a complex solution by extracting it as the yellow bromothymol blue salt in the presence of acetate buffer pH 5.0, using toluene as solvent. A somewhat similar method was described several years ago for quinine, using eosin as the dye anion (*9, 10*).

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Determination of Vitamin B_1 (Thiamine) in Extracts and Concentrates

Comparison of Biological and Chemical Methods

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IT IS the purpose of this paper to correlate the data obtained during the past several years, in determining thiamine by six different procedures. The following comparisons have been made:

1. The modified Smith-curative and modified Melnick-Field methods with the U. S. P. method.
2. The rat-growth and pigeon-weight-maintenance methods with the modified Smith-curative method.
3. The thiochrome method of Hennessy and Cerecedo with the modified Melnick-Field method.

TABLE I. COMPARISON OF U. S. P., MODIFIED SMITH-CURATIVE, AND MODIFIED MELNICK-FIELD METHODS, USING THIAMINE SOLUTIONS

Sample No.	U. S. P.	Modified Smith-Curative	Modified Melnick-Field	Difference[a] Smith	Difference[a] Melnick-Field
	γ/ml.	γ/ml.	γ/ml.	%	%
42,200	1,100	1,100	1,140	0.0	+3.5
41,680	6,138	6,600	6,600	+7.5	+7.5
37,050	6,600	6,600	6,606	0.0	+0.1
42,650	11,889	10,998	11,367	−7.5	−4.4
37,370	24,000	24,000	27,240	0.0	+13.5
41,340	25,944	26,178	26,100	+0.9	+0.6
42,440	25,998	28,164	28,236	+7.2	+8.6
42,640	28,100	25,998	26,700	−7.5	−5.0
44,490	55,812	57,230	58,632	+2.4	+5.1
42,450	60,000	64,980	63,978	+8.3	+6.6
42,610	64,863	60,000	62,508	−7.5	−3.6
Mean (11)....		....	....	4.4	5.3

[a] Expressed as difference in per cent of value obtained by U. S. P. method.

Description of the Methods

BIOLOGICAL METHODS. The U. S. P. method was carried out as described in the U. S. Pharmacopoeia XI (*10*). The rat-curative method was that described by Smith (*9*) and modified by Birch and Harris (*2*), so as to carry 0.5 per cent of yeast. This small amount of yeast greatly increased the number of rats which developed typical polyneuritis and at the same time it did not influence the response to treatment. The rat-growth method was essentially that described by Chase and Sherman (*3*). The pigeon-weight-maintenance method was that of Cowgill (*4*).

CHEMICAL METHODS. The colorimetric method of Melnick and Field (*8*) as modified by Emmett, Peacock, and Brown (*5*) was used. This was further improved by introducing three changes: separation of the colored reaction product on Super-Filtrol by centrifuging, eluting the color with 10 cc. of 95 per cent ethyl alcohol, and measuring the depth of color with the Evelyn colorimeter, using filter No. 520. The thiochrome method of Hennessy and Cerecedo (*6*) was used along with the adoption of Mason and Williams' (*7*) very simple and efficient procedure for the oxidation and extraction of the thiochrome with isobutanol, and measuring the fluorescence with the Pfaltz and Bauer fluorophotometer.

Results and Discussion

COMPARISON OF THE U. S. P., MODIFIED SMITH-CURATIVE, AND MODIFIED MELNICK-FIELD METHODS. In Table I the data obtained in assaying 11 different samples of thiamine, with potencies ranging from 1100 to 65,000 micrograms per ml., are given. The concordance of the results for the corresponding samples assayed by the three methods is very good. With but one exception the data agree within ±9 per cent of the values of the U. S. P. method—the mean differences being 4.4 for the rat-curative method and 5.3 per cent for the colorimetric method.

Table II gives a summary of these three methods as applied to 76 samples, which include pure thiamine, elixirs, synthetic vitamin mixtures, wheat germ extracts, fortified liver extracts, and miscellaneous samples. The thiamine solutions and the elixirs gave the best results. The vitamin mixtures were made up of additional components of the vitamin B complex, supplied either in crystalline form or as natural concentrates. In addition, some of them contained the fat-soluble vitamins A and D. The variations between the three methods with these samples are somewhat higher than for pure thiamine and elixirs, but still within the range of accuracy of the biological assays. The correlation between the data for the three methods in the case of the wheat germ and liver extracts is satisfactory. The miscellaneous samples, which included rice-bran concentrates, dried yeast, thiamine hydrochloride tablets, and alcoholic solutions of vitamin B complex mixtures, show the greatest discrepancies.

From these data it is evident that any one of these three methods will give satisfactory results. The Smith-curative method has a distinct advantage, however, over the U. S. P. procedure in that it is more rapid. In turn, the chemical method has an advantage over the two biological procedures as to time.

COMPARISON OF THE MODIFIED SMITH-CURATIVE, RAT-GROWTH, AND PIGEON-WEIGHT-MAINTENANCE METHODS. In Table III data are presented for 10 samples of wheat germ extract and 10 of yeast concentrates, bioassayed by three methods. The mean values obtained agree within 10 to 15 per cent, but the rat-growth and pigeon-weight-maintenance methods gave slightly higher results than the curative methods. This may be due to a partial deficiency in the rat-growth and pigeon-weight-maintenance basal diets, which was probably corrected by B vitamin factors

TABLE II. COMPARISON OF U. S. P., MODIFIED SMITH-CURATIVE, AND MODIFIED MELNICK-FIELD METHODS

Type of Sample	No. of Samples	Range of Values of Thiamine Content: U. S. P.	Modified Smith-curative	Modified Melnick-Field	Mean Difference[a] Smith	Mean Difference[a] Melnick-Field
		γ/ml.	γ/ml.	γ/ml.	%	%
Thiamine solutions	11	1,100–64,863	1,100–60,000	1,140–62,508	4.4	5.3
Elixirs	7	150–31,410	156–31,410	159–33,750	1.4	6.8
Vitamin mixtures	11	9–13,140	8–11,499	6–11,328	7.3	10.5
Wheat germ extracts	24	90–201	87–216	87–231	8.8	12.6
Liver extracts fortified	14	17–26,667	15–26,253	13–25,500	4.1	7.9
Miscellaneous	9	42–43,778	51–37,562	48–39,072	13.5	14.6

[a] Expressed as difference in per cent of value obtained by U. S. P. method.

present in the wheat germ extracts and the yeast concentrates. With certain types of materials of low potency the curative methods are not suitable because of the inherent difficulty of giving large amounts of samples orally to polyneuritic animals. Obviously then, for this type of sample, it is necessary to use the rat-growth or the pigeon-weight-maintenance methods.

Comparison of the Modified Melnick-Field and Thiochrome Methods. The values obtained with these two methods are given in Table IV. An inspection of the results shows a very good agreement. In general, however, the values obtained by the thiochrome method were slightly lower than those found with the colorimetric method except in the case of the samples of thiamine (at the top of the table) which contained little or no interfering material.

In the course of many assays, carried out in this laboratory by the colorimetric method, the authors have found the reproducibility of values obtained on any given sample to be very good, while with the thiochrome method this has not always been the case. With the thiochrome method a repeat assay will occasionally yield a value 15 to 20 per cent different from the original value for no apparent cause. Whether these variations are primarily due to faults in the chemical procedure or to errors incurred in the measurement of the fluorescence has not as yet been definitely determined. In this laboratory, the colorimetric method has definitely proved to be the more reliable of the two. It is, however, less sensitive in that it requires a concentration of 2 to 3 micrograms per milliliter while the thiochrome method can be carried out on a concentration of as little as 0.05 microgram of thiamine per milliliter. Further, in the case of very potent samples, the base exchange step is unnecessary because the samples can be diluted to the point where interference by foreign material is negligible. However, in cases where the base exchange procedure must be employed, the thiochrome method has no advantage over the colorimetric procedure with respect to time.

In general the fluorometric method has been used much more extensively than the Melnick-Field method. The possibility of adapting the latter for the determination of thiamine in samples of low potency has not been thoroughly studied. The method has, however, recently been adapted to urine by Alexander and Levi (*1*), and should therefore be of particular interest to laboratories which are not equipped for measuring fluorescence.

Table III. Comparison of Modified Smith-Curative, Growth, and Pigeon-Weight-Maintenance Methods

Sample No.	Thiamine Content: Modified Smith-curative	Rat-growth	Pigeon-weight-maintenance	Mean Difference[a]: Growth	Weight maintenance
	γ/g.	γ/g.	γ/g.	%	%
		Wheat Germ Extracts			
95,647	66	90	81	+36.4	+22.7
93,337	69	75	78	+8.7	+13.0
94,287	72	75	72	+4.2	0.0
93,567	75	75	78	0.0	+4.0
95,507	75	90	81	+20.0	+8.0
93,967	78	99	60	+26.9	−23.1
95,197	78	90	81	+15.4	+3.9
95,797	78	90	90	+15.4	+15.4
92,716	90	75	102	−16.7	+13.3
94,907	90	90	75	0.0	−16.7
Mean (10)	...	...	...	14.4	12.0
		Yeast Concentrate			
94,537	750	750	750	0.0	0.0
95,807	750	900	900	+20.0	+20.0
96,897	750	825	669	+10.0	−10.8
95,417	771	771	849	0.0	+10.1
97,097	800	800	600	0.0	−25.0
92,977	831	999	999	+20.2	+20.2
93,797	855	999	900	+16.8	+5.3
94,547	900	900	900	0.0	0.0
95,917	900	999	981	+11.0	+9.0
97,087	999	800	750	−19.8	−25.0
Mean (10)	...	...	...	9.8	12.5

[a] Expressed as difference in per cent of value obtained by modified Smith-curative method.

Table IV. Comparison of Modified Melnick-Field and Thiochrome Methods

Sample		Thiamine Content: Modified Melnick-Field	Thiochrome	Difference[a]
		γ/ml.	γ/ml.	%
3,422	Thiamine solutions	5,350	5,600	+4.7
7,962		23,600	24,800	+0.5
9,542		55,400	56,100	+1.3
92,982		57,300	62,000	+8.2
7,072		111,700	109,000	−2.4
5,562	Elixir	231	237	+2.6
59,471		99	105	+6.1
4,092	Wheat germ extract	191	181	−5.2
94,472-A	Yeast extract	306	282	−7.8
94,472-B		348	336	−3.4
99,642	Liver extract fortified	14.4	14.0	−2.8
1,592	Vitamin mixture (ABCD)	2,450	2,290	−6.5
		γ/g.	γ/g.	
65,881		8,200	7,150	−12.8
70,652		8,200	7,750	−5.8
74,002		8,100	7,400	−8.6
88,432		8,900	9,000	+1.1
99,582	Vitamin B complex	4,010	3,880	−3.4
99,592		3,900	3,810	−2.3
5,572		3,900	3,630	−6.9
98,482	Ration supplement	4,000	3,640	−9.0
Mean (20)		.....	.....	5.1

[a] Expressed as difference in per cent of values obtained by modified Melnick-Field method.

Summary

The choice of a method for the determination of thiamine will be governed, in the main, by the potency of the samples to be assayed. From the standpoint of pharmaceutical preparations, the modified Smith-curative method is preferable to the U. S. P., rat-growth, or pigeon-weight-maintenance methods because of the rapidity of obtaining results. However, if the samples are low in potency, it is necessary to employ the rat-growth or the pigeon-weight-maintenance method, because of the difficulty of administering large doses to a polyneuritic rat.

Good agreement was found between the values obtained with the modified Melnick-Field and the thiochrome methods. The modified Melnick-Field method has proved to be the more reliable of the two, but the thiochrome method is more sensitive and is, therefore, the method of choice for samples of very low potency.

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Sugar Determination in Starch Hydrolyzates

By Yeast Fermentation and Chemical Means

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THE quantitative analysis of mixtures containing dextrose, maltose, and dextrins resulting from starch decomposition is one of the most difficult problems in organic analytical chemistry. A majority of the analytical methods are based upon the chemical reducing power of these hydrolyzed starch products, and are subject to certain errors due to lack of specificity. In the last two decades, however, methods have been devised which utilize the remarkable selectivity exhibited by certain cultures of yeast and bacteria in respect to their fermentative action upon sugars (*3*, *4*, *7*, *9*, *11*). These biological methods appear to be more specific than any purely chemical procedure. A direct comparison between biological and chemical analyses has not heretofore been made. In the present communication the authors have attempted such a comparison in the determination of dextrose and maltose in a series of commercial starch hydrolyzates and in the successive steps of an acid hydrolysis of starch. The latter work showed that certain sugar curves which have stood for 45 years as a classic picture of the relative composition of starch solutions during acid conversion may be in error.

The biological method used was developed at this laboratory, and depends upon the measurement of carbon dioxide evolved during fermentation of sugar by yeast. The chemical method chosen is of the copper reduction type which Sichert and Bleyer (*8*) adapted for the analysis of commercial starch sirups and malt extracts.

Biological Method

The yeast fermentation method used is based upon the procedure outlined by Schultz and Kirby (*7*), who determined fermentable sugars by measuring carbon dioxide evolved. Measurements are made with the fermentometer designed by Schultz, Atkin, and Frey (*6*), which consists essentially of a series of twelve 120-ml. wide-mouthed reaction bottles connected by means of pressure tubing to twelve 260-ml. gas burets or "gasometers". The bottles are shaken at 110 cycles per minute in a cradle immersed in a 30° C. constant-temperature water bath. Fleischmann enriched baker's yeast is used to ferment the common sugars—i. e., dextrose, fructose, sucrose, and maltose. A special yeast, Fleischmann Hi-B_1 No. 2019, is used to ferment all the above sugars except maltose. The difference in the total sugars fermented by the two yeasts therefore represents maltose. That part of the dextrin or starch subject to attack by β-amylase may be determined by adding a source of β-amylase such as soy, barley, or wheat flour to the baker's yeast fermentation.

Subtracting from the total fermentables thus obtained the quantity of dextrose and maltose previously determined in the original product, one is provided with a measure of the "β-amylase-attackable substances".

Each bottle, before the addition of sugar and yeast, contains the following yeast nutrients:

0.5 ml. of $MgSO_4.7H_2O$ (150 mg. per ml.)
0.5 ml. of KCl (100 mg. per ml.)
2.5 ml. of phosphate buffer (pH 6.0 to 6.5) containing per liter:

180 grams of $NH_4H_2PO_4$
72 grams of $(NH_4)_2HPO_4$
0.2 gram of nicotinic acid

Two bottles are used for a "standardization curve", one containing 0.5 gram of C. P. grade dextrose, the other 1 gram. An amount of the unknown which contains between 0.5 and 1 gram of fermentable sugar is weighed or pipetted into another reaction bottle. If the unknown is low in sugar, 0.5 gram of dextrose may be superimposed upon it. The total volume in each bottle should now be adjusted to 40 ml. by the addition of distilled water. When these preparations are complete, 10 ml. of a 10 per cent suspension of yeast are pipetted into each bottle. The bottles are then immediately connected to the gasometers. Fermentation is assumed to be complete when no more carbon dioxide is evolved during a 15-minute period. The difference between the amounts of carbon dioxide produced by 0.5 and 1 gram of pure dextrose is used to calculate the sugar content of the unknown. Dextrose is used as a standard throughout because it has been established that the fermentation of maltose hydrate or dextrose by baker's yeast will produce identical volumes of carbon dioxide.

Chemical Method

Barfoed (*1*) in 1873 formulated a copper acetate reagent that is not reduced to any great extent by disaccharides. This reagent was adapted for quantitative use by Steinhoff (*10*), who substituted sodium acetate for acetic acid and estimated cuprous oxide volumetrically with iodine and thiosulfate. In the present work, the authors have used Sichert and Bleyer's (*8*) modification of Steinhoff's method, worked out for the analysis of acid or enzymically hydrolyzed starch products. In brief, to determine dextrose, they reduce copper held in an acetate complex in a sugar solution by heating the reagent beaker for 20 minutes in a boiling water bath. Precipitated cuprous oxide is filtered on an asbestos mat in a Gooch crucible. After being washed with hot water (60° to 80° C.), the mat is transferred to the reaction beaker and stirred in an acidified ferric alum solution until the oxide is completely dissolved. The ferrous ion produced by reaction with cuprous oxide is titrated with standardized 0.1 N potassium permanganate. Dextrose and maltose are determined together as dextrose equivalent with the more alkaline Fehling's solution in a similar manner. Milligrams of dextrose, value A, equivalent to milliliters of 0.1 N potassium permanganate can be found in Sichert and Bleyer's copper acetate table. The dextrose equivalent, value B, is given by Sichert and Bleyer's Fehling's solution table. Thus maltose $= 2(B - A)$.

In place of the boiling water bath, the authors found it more convenient to use an Arnold steam sterilizer, adjusting the time of heating to 11 minutes by testing with pure sugars, so that they could use Sichert and Bleyer's own tables.

TABLE I. ASSAY OF COMMERCIAL STARCH HYDROLYZATES

Product	Total Sugar, Fermentation, Yeast I[a]	Dextrose Fermentation, yeast II[b]	Dextrose Chemical, copper acetate	Maltose Hydrate Fermentation	Maltose Hydrate Chemical	Maltose Hydrate Difference	β-Amylase Convertible, Fermentation
	%	%	%	%	%	%	%
A Malt sirup	60.8[c]	5.8	6.1	55.0	56.3	1.3	1.9
B Acid and malt sirup	60.0	29.6	28.8	30.4	32.4	2.0	6.7
C Malt sirup	55.0	2.7	4.2	52.3	56.5	4.2	7.6
D Corn sirup	34.8	16.9	17.5	17.9	32.1	14.2	28.7
E Dried low-conversion malt sirup	13.8	2.8	3.9	11.0	23.0	12.0	36.5
F Dried corn sirup	38.0	18.6	19.8	19.4	35.0	15.6	38.5
G Dried low-conversion malt sirup	16.3	6.3	6.9	10.0	24.4	14.4	56.3

[a] Fleischmann enriched baker's yeast, ferments common sugars.
[b] Fleischmann Hi-B_1 No. 2019, ferments common sugars, but not maltose.
[c] All products analyzed on "as is" basis.

Experimental

A group of seven commercial starch hydrolyzates which are in common use in the baking industry was analyzed by both the yeast fermentation and chemical methods. The results of these assays are presented in Table I.

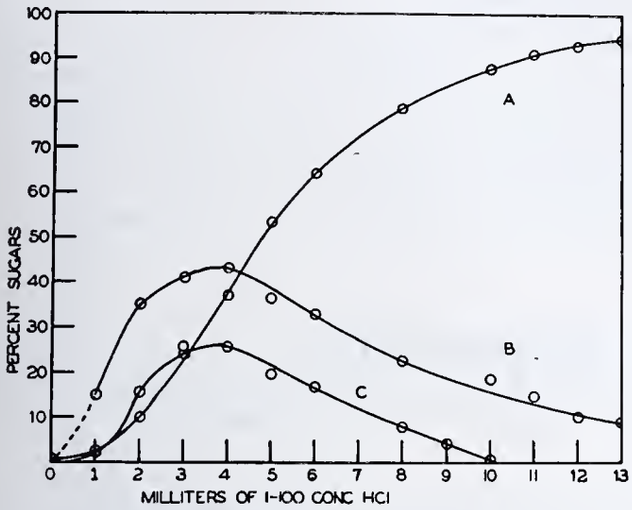

FIGURE 1. ACID HYDROLYSIS OF CORNSTARCH
Per cent sugars on dry basis of starch
A. Dextrose by yeast fermentation method
B. Maltose by chemical method
C. Maltose by yeast fermentation method

The data on the acid hydrolysis of starch given in Figure 1 were obtained in the following manner:

A group of 100-ml. volumetric flasks, each containing 5 grams of cornstarch suspended in 50 ml. of distilled water to which varying amounts of dilute hydrochloric acid had been added, was autoclaved for 2 hours at a pressure 9.1 kg. (20 pounds) in excess of atmospheric pressure. When the suspensions had been cooled, neutralized, and diluted to volume, they were analyzed for dextrose and maltose by each of the two methods described.

Discussion

The results of analyses of commercial starch hydrolyzates (Table I) by chemical and by fermentation methods do not agree, particularly in the determination of maltose. The difference is evidently due to the interference of malto-dextrin, or reducing dextrin, formed by the incomplete hydrolysis of starch. Fehling's solution cannot be used to distinguish maltose or dextrose from these other reducing substances. One may obtain a measure of the quantity of such reducing dextrins in a product by fermenting it with baker's yeast in the presence of β-amylase, as previously described. A comparison of columns 4 through 7 in Table I will show a general relationship between the amount of starch or dextrin which can be converted to fermentable sugar by β-amylase and the reducing power these substances are able to exert upon Fehling's solution. The difference between the results of the two methods for product A is 1.3 per cent and the amount of β-amylase-convertible starch is 1.9 per cent. In product G, however, in which the β-amylase-convertible starch is 56.3 per cent, the difference between the two methods rises to 14.4 per cent.

The chemical method also tends to produce higher values for dextrose and this may be attributed to the slight reducing action caused by maltose on the copper acetate reagent. This is especially noticeable when maltose is present in large quantities.

Sichert and Bleyer's chemical method is claimed to be adequate for use in analyzing products of starch decomposition. It is satisfactory for this purpose, however, only under limited conditions—namely, when the product under consideration contains a very small proportion of reducing dextrin. Sichert and Bleyer (*8*) analyzed a sirup of this type, and found that it contained 89.5 per cent dextrose (dry basis) and only 1.7 per cent maltose and 1.3 per cent dextrin. This is obviously not typical of a commercial starch sirup.

For many years it has been customary to use the term "maltose" to describe both maltose and dextrins which reduce Fehling's solution, and also mixtures of the two. Whenever the analytical method is not specific for maltose, some qualifying phrase should be used, such as "maltose equivalent", "maltose plus malto-dextrins", etc. This distinction should be emphasized as much as possible because the use of "maltose" alone may be misleading. The availability of methods specific for maltose makes anything else obsolete.

Rolfe and Defren (*2*, *5*) produced curves designed to describe the composition of a starch mixture in terms of dextrose and "maltose" at any time during acid conversion (Figure 2). They determined sugars by a combination of copper reduction and polarimetric methods, which apparently could not distinguish between true maltose and reducing dextrins. The results of the authors' attempt to reproduce Rolfe and Defren's curves are shown in Figure 1. Since the data obtained for dextrose content were nearly identical for both methods, only the fermentation curve for this sugar is reproduced. The "maltose" maximum attained by chemical analysis of the authors' starch conversion series was 43 per cent which is lower than Rolfe and Defren's maximum of 47 per cent, but close enough to show the similarity between the two methods used. The contrast between these maxima obtained by chemical means and the highest point on the true

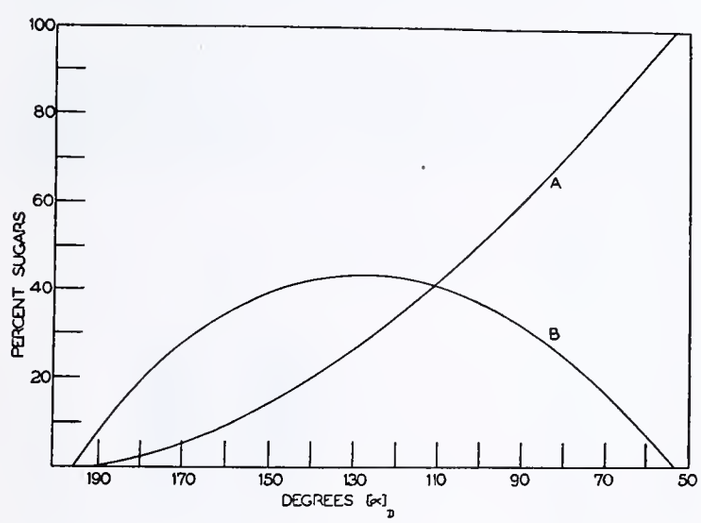

FIGURE 2. ACID HYDROLYSIS OF STARCH BY ROLFE AND DEFREN (*5*)
Per cent sugars on dry basis of starch by chemical-polarimetric method
A. Dextrose
B. Maltose

maltose curve, 25.2 per cent as determined by fermentation analysis, is a graphic demonstration of the wide difference between the two types of methods. This is in substantial agreement with work done in 1925 by Nanji and Beazeley (*4*), who, using a combined yeast fermentation–specific rotation method of their own, obtained a maximum of 29 per cent maltose in analyzing a potato starch conversion.

Summary

A number of commercial starch hydrolyzates were analyzed for dextrose and maltose by measuring carbon dioxide

produced by yeast fermentation, and by the Sichert and Bleyer chemical method. Both methods gave satisfactory results in determining dextrose, but only the biological method, because of its high specificity, could be relied upon in analyzing for maltose. The chemical method is unreliable for the determination of maltose in the presence of the reducing dextrins which occur in both acid and enzyme-hydrolyzed starch products. β-Amylase can be used to give an indication of the amount of such reducing dextrins.

The two methods applied to the analysis of the course of acid hydrolysis of starch showed that the longstanding work of Rolfe and Defren is in error, owing to the lack of specificity of Fehling's solution for the determination of maltose in the presence of reducing dextrin.

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Gas-Absorption Apparatus

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DURING a series of acetylation studies with ketene it became necessary to devise a compact, flexible, and efficient type of absorption apparatus that would provide intimate and prolonged contact of the gas with the liquid reactant. After testing several arrangements of the traditional absorption equipment, the piece of apparatus shown in Figure 1 was constructed and has been found to be thoroughly satisfactory. The arrangement can be used for the absorption of other gases under similar conditions. The use of the glass-bead column was suggested by Shaw (*1*) but this equipment is sufficiently different to justify description.

A 6-cm. piece of 6-mm. glass tubing, *A*, is sealed endwise to a 10-cm. piece of 12-mm. glass tubing, *B*. Four small oblong holes, *C*, of approximately 4-mm. diameter, are blown in the larger tube just above the point of sealing. A 6-cm. length of 20-mm. glass tubing, *D*, is then sealed to *B*, just above the four openings. *D* should not reach to the lower level of *A* by some 1 or 2 cm., in order to provide the correct circulating operation when the completed equipment is in use. This arrangement provides two concentric tubes, *A* and *D*, with *B* extending above the other two as shown. The upper end of *B* should be attached to an efficient reflux condenser during operation, by means of a rubber or ground-glass connection. The space between *A* and *D* is then nearly filled with solid glass beads and enough constrictions are formed at the lower end of *D* to hold the glass beads in place. *B* is inserted through the opening of a one-hole stopper that closes a 125-ml. filtering flask, *E*. The accompanying illustration shows all these essential features. Proportionate changes in dimensions may be made, so that this equipment can be used with any available size or type of filtering flask.

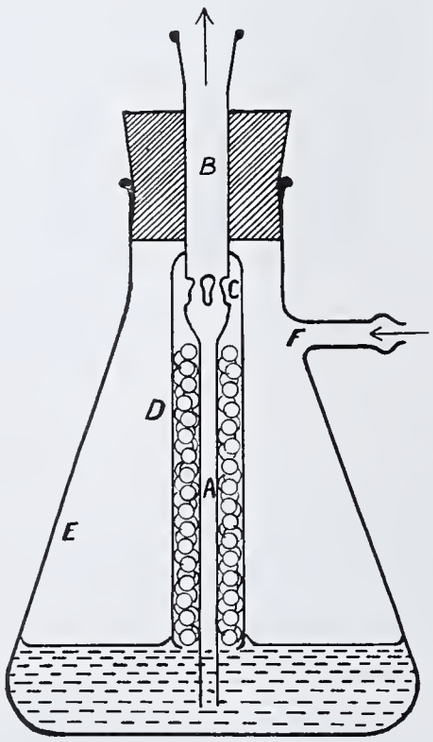

FIGURE 1

The gas to be absorbed is then introduced through the side arm, *F*, of the filtering flask. The lower end of *D* should dip just below the surface of the liquid absorbent. As the pressure within the flask increases, the liquid is forced up *A* and *D*, until a few bubbles enter the glass-bead column. Here a percolating effect is produced, as gas and liquid ascend through the glass beads. A large portion of the resulting solution returns through *A*. As the volume of the liquid increases, owing to the absorption of gas, the same relative positions of liquid to absorption apparatus may be maintained by raising the glass tubes through the supporting stopper.

This piece of equipment differs materially from that designed by Shaw (*1*) in the following major respects. It was constructed to handle relatively large volumes of liquids or solutions and highly concentrated gases in small volumes, rather than small volumes of liquids and large volumes of dilute gases. It has been employed in the preparation of numerous organic acetates in yields of better than 90 per cent of the theory, and in quantities of better than 40 grams of the final acetate. The absorption apparatus has operated continuously for 20 hours or more without further adjustment, except the raising of the absorption tube in relation to the liquid in the filtering flask. The rate of flow of ketene was 0.07 mole per hour (3 to 4 liters of mixed pyrolysis gases), although any slower perceptible rate of flow would be absorbed with the same quantitative efficiency. The average back pressure during operation is 50 to 60 mm. of water, which is readily counterbalanced by any closed traditional gas generator.

A further advantage of this equipment is that precipitates, produced by reaction between gas and liquid, do not clog or hinder the operation, since there are no small openings to become clogged. In case the gases employed are less soluble than ketene, the length of the glass-bead column may be extended to permit longer contact between the gas and liquid solvent. The entire piece of equipment is relatively cheap and can be constructed by any efficient glass blower from materials readily available in any laboratory.

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Determination of Tetraethyllead in Gasoline

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A procedure for the rapid determination of tetraethyllead in gasoline, based on modifications of the Baldeschwieler (*2*) nitric acid method, is described. By using 40 per cent nitric acid containing 6 per cent potassium chlorate and a separatory funnel, complete extraction of the lead is effected in 8 minutes with clean-cut separations and negligible heat rise even with 100 per cent cracked gasolines. The amount of lead is determined in the extractions by either volumetric or gravimetric methods. When frequent determinations are to be made, a preferred volumetric chromate method is recommended as more rapid than the gravimetric methods described.

THE production of maximum quantities of high-octane aircraft engine fuels necessitates the use of tetraethyllead to the top limits of the permissible tetraethyllead content. To facilitate the commercial blending of gasolines to these top limits, an accurate and rapid method for the determination of tetraethyllead is required.

The present A. S. T. M. method (*1*) and the nitric acid extraction method proposed by Baldeschwieler (*2*) require from 3 to 4 hours to complete a lead determination. The number of tests that can be run at one time by the A. S. T. M. method is also limited by the extractors available. The disadvantages of these and other proposed methods are the time required for extraction and the necessity of oxidizing organic matter which is extracted with the lead. The possibility of loss of lead in extraction at higher temperatures is recognized in provision for the use of a heavy solvent in the A. S. T. M. method.

This paper describes an extraction procedure and lead determination methods which overcome the disadvantages of previous methods. The total time of test is usually less than one hour. Clean-cut separations in the extraction are obtained almost instantaneously, complete removal of the lead is effected within 8 minutes, and without further oxidation of organic matter the lead may be completely precipitated as chromate.

The method is a modification of Baldeschwieler's nitric acid extraction procedure (*2*), replacing concentrated nitric acid with a 1.2 to 1 nitric acid solution saturated with potassium chlorate and containing a trace of copper nitrate. The extracted lead is determined by a volumetric oxidation-reduction reaction after precipitation as lead chromate.

Preparation of Extraction Solution

Measure 550 ml. of concentrated nitric acid (specific gravity 1.42) into a tall 1000-ml. G. G. S. cylinder, introduce 78 grams of potassium chlorate, stopper securely, and shake vigorously until most of the potassium chlorate is dissolved. Add 450 ml. of water and shake again until solution is complete. Dissolve 0.1 gram of sheet copper in a small beaker with a minimum of nitric acid and evaporate to sirupy consistency. Dissolve the contents with a little of the extraction solution and rinse into the main portion. Mix until uniform. A solution so prepared will have a specific gravity of 1.30 at 21° C. (70° F.) and will contain approximately 40 per cent of nitric acid by weight and 6 per cent by weight of potassium chlorate.

NOTE: A private communication from W. M. Basch, Standard Oil Development Co., indicates that for gasolines rich in unsaturates better results were obtained in his laboratory by adding an excess of potassium chlorate to the nitric acid solution and allowing the mixture to stand approximately 5 to 7 days before using.

Procedure

Add 15 ml. of the nitric acid–potassium chlorate solution to 100 ml. of gasoline in a 500-ml. separatory funnel. Stopper securely and shake gently at first, releasing the pressure frequently, then shake more vigorously for 3 minutes until no further reaction is noticeable and the gasoline layer becomes perfectly clear. Allow to settle for 1 minute, then draw off the acid layer into a 500-ml. Erlenmeyer flask. Repeat the extraction twice, reducing the shaking periods to 1 minute.

From this point the lead may be determined by any standard procedure for lead determination, but methods permitting greater speed without sacrifice of accuracy are to be preferred. Descriptions of the volumetric and gravimetric chromate methods and of the lead sulfate method are presented because of modifications which speed up the determination and maintain its accuracy. This laboratory recommends the volumetric chromate method, when frequent determinations are necessary, as it is the most rapid.

VOLUMETRIC DETERMINATION AS CHROMATE. Evaporate the combined extracts just to crystallization (do not take to complete dryness). Add about 100 ml. of hot water and heat to boiling. Allow to cool for a few seconds. Neutralize with ammonium hydroxide to the characteristic deep blue of the cuprammonium compound and add 2 ml. in excess. Acidify with acetic acid and add 1 ml. in excess. Bring to a boil and add 10 ml. of 5 per cent potassium dichromate solution. Digest on the hot plate for 7 minutes. Filter through a Gooch crucible and wash thoroughly with hot water, then with three portions of about 5 ml. each of acetone, followed by a similar ethyl ether wash to remove organic matter which may impede the subsequent solution of the precipitate. Dry for a few minutes at 100° C. until no odor of ether is noticeable.

Transfer the Gooch crucible to a clean 500-ml. suction flask. Dissolve the lead chromate, using suction, with 150 ml. of a solution obtained by adding 200 ml. of hydrochloric acid (specific gravity 1.19) and 350 ml. of water to 1 liter of filtered saturated commercial sodium chloride solution. Make up to 250 ml. with cold water. Introduce 2 grams of solid potassium iodide crystals, gently swirl the flask to dissolve the potassium iodide, and titrate the liberated iodine immediately with 0.04 *N* sodium thiosulfate solution until the yellow color due to the free iodine is very faint. Then add 2 ml. of starch solution and complete the titration to a clear green end point.

GRAVIMETRIC DETERMINATION AS CHROMATE. Where gravimetric determination is desired, proceed as above through digestion on the hot plate. Filter through a tared Gooch crucible and wash thoroughly with hot water, then with three 5-ml. portions of acetone, followed by a similar ethyl ether wash. Dry for a few minutes at 100° C. until no odor of ether is noticeable. Cool and weigh.

GRAVIMETRIC DETERMINATION AS SULFATE. If it is desired to determine the lead gravimetrically as lead sulfate, draw off the extract into a 400-ml. beaker instead of an Erlenmeyer flask, add 9 ml. of concentrated sulfuric acid to the combined extracts in the beaker, and evaporate to fumes on a hot plate. If any charring occurs add cautiously (one drop at a time to avoid spattering) concentrated nitric acid which is saturated with potassium chlorate until no trace of charring is observed when brought to copious fumes. Allow to cool, add about 5 ml. of cold water, and evaporate again to the fuming point. Allow to cool for about 2 minutes, add 70 ml. of lead acid (*3*), and heat to boiling. The lead acid is a dilute sulfuric acid saturated

Table I. Time Required for Complete Extraction of Lead

	Time of Shaking	Tetraethyllead Extracted
	Min.	*Ml./gal.*
1st Shaking	3	3.76
2nd Shaking	1	0.18
3rd Shaking	1	0.01
4th Shaking	1	0.00[a]
Total	6	3.95

[a] Complete removal of lead was effected in three extractions on all samples tested.

with lead sulfate. Filter through a tared Gooch crucible as soon as the solution is cooled to room temperature. Wash thoroughly with the lead acid, then with three 5 ml. portions of acetone, followed by a similar ethyl ether wash. Dry for a few minutes at 100° C. until no odor of ether is noticeable. Weigh when cool.

Standard Lead Nitrate Solution

In the determination of lead by the volumetric chromate method it is customary to standardize the thiosulfate solution accurately against pure lead nitrate. The method used in this laboratory is as follows:

Dissolve 7.1808 grams of C. P. lead nitrate in water containing 5 ml. of nitric acid and make up to exactly 1 liter in a volumetric flask. For standardization of a sodium thiosulfate solution (preferably 0.04 *N*), pipet 25 ml. of the standard lead nitrate solution into a 500-ml. Erlenmeyer flask. Add 45 ml. of the nitric acid–potassium chlorate solution and evaporate to crystallization under a hood. Remove from the heat, add 100 ml. of hot water, and finish the test exactly as described for the volumetric determination.

On the basis of 100 ml. of gasoline taken for a test, 25.0 ml. of the standard lead nitrate solution are equivalent to 4.00 ml. of tetraethyllead per gallon. The calculation would therefore be:

$$\text{Ml. of } Na_2S_2O_3 \times K = \text{ml. of tetraethyllead per gallon}$$

$$\text{where } K = \frac{4.00}{\text{ml. of } Na_2S_2O_3}$$

The sodium thiosulfate solution should age for at least one week before standardization and should be checked about once each month.

Table II. Typical Comparative Results

Gasoline	Acid Heat	Tetraethyllead by A. S. T. M. Method[a]	Tetraethyllead by Nitric Acid–Chlorate Method: Chromate volumetric	Chromate gravimetric	Sulfate gravimetric
	° *F.*	*Ml./gal.*	*Ml./gal.*	*Ml./gal.*	*Ml./gal.*
A	100	0.25	0.25	0.26	0.26
B	111	2.60	2.61	2.62	2.62
C	24	2.99	3.00	3.00	2.99
D	1	3.99	3.99	4.00	4.01
Average time for test, hours		3–4	0.5	0.75	1

[a] Extracted and weighed as lead chromate in accordance with A. S. T. M. method (*1*).

Discussion

While 1.2 to 1 nitric acid solution saturated with potassium chlorate was found to be best suited to the average type of gasoline tested in this laboratory, stronger or weaker solutions may be more advantageous, depending upon the particular type of gasoline most frequently tested. When employing a stronger solution on straight-run gasolines the initial shaking period of 3 minutes can be reduced considerably. However, a concentration weaker than one volume of acid to one volume of water will necessitate a longer period for the first shaking. An 0.9 to 1 solution of nitric acid saturated with potassium chlorate will take about 10 minutes for the first shaking.

With 100-octane and similar gasolines, which are colored green or blue, the breaks between the gasoline and acid layer are so clean-cut that it is sometimes difficult to see the line of separation. In such cases it is advisable to add 1 drop of oil-soluble red dye. The red dye is stable, while the green and blue dyes fade.

The time required for complete decomposition and extraction has been determined on a sample of 100-octane gasoline containing 3.95 ml. of tetraethyllead per gallon with results as shown in Table I.

The time required for a completed lead determination has been reduced from 4 hours to less than 1 hour. The important contributing factors are:

1. The improved extraction method reduces the time for complete removal of the lead to 8 minutes as compared with 1 hour by the hydrochloric acid extraction or 1.5 hours by the Baldeschwieler method. Further time is saved, owing to the smaller volume of extract to evaporate (approximately 45 ml. in this method, 150 ml. in the hydrochloric acid method, and 80 ml. in the Baldeschwieler method).
2. The use of the thiosulfate titration eliminates the drying, ignition, and weighing of precipitates necessary in the usual gravimetric methods.

Table III. Heat Rise of Cracked Gasolines

Gasoline	Acid Heat	Gasoline Temperature before Shaking	Gasoline Temperature after Shaking: After 1st shaking	After 2nd shaking	After 3rd shaking
	° *F.*	° *F.*	° *F.*	° *F.*	° *F.*
F[a]	120	60	70	70	70
G[b]	120	71	80	79	78
K[c]	88	76	92	89	88
L[d]	97	75	91	88	84

[a] Unleaded catalytically cracked gasoline.
[b] Prepared by adding 3 ml. of tetraethyllead per gallon to gasoline F.
[c] Cracked gasoline, containing 0.54 ml. of tetraethyllead per gallon.
[d] Cracked gasoline containing 2.98 ml. of tetraethyllead per gallon.

Time-saving is also apparent where this extraction method is used in conjunction with the modified gravimetric methods described. In the gravimetric chromate method, use of the acetone-ether washing eliminates the necessity of complete oxidation of organic matter and lessens the drying time. In the sulfate method, complete oxidation of all organic matter is obtained more rapidly because of the potassium chlorate. The use of alcohol and one-hour settling in an ice bath is unnecessary when lead acid (*3*) is substituted for dilution and washing. The acetone-ether wash with drying at 100° C. replaces the ignition of the lead sulfate.

Table II, typical of several hundred tests run in this laboratory, shows that accuracy and reproducibility have been maintained.

Safety of Potassium Chlorate in Dilute Nitric Acid

Potassium chlorate in dilute nitric acid solution can be used safely when extracting cracked gasolines; the actual temperature rise resulting is given in Table III. The results obtained in this experiment indicate that the heat rise due to shaking a cracked gasoline with the extraction solution is not necessarily in direct proportion to the acid heat. In no case, however, is the heat so generated sufficient to interfere with the procedure.

Table IV. Influence of Small Amounts of Copper

Determination	Standard $Pb(NO_3)_2$ Equivalent to:	Results with Copper Indicator: Volumetric chromate method	Gravimetric chromate method	Gravimetric sulfate method
	Ml./gal.	*Ml./gal.*	*Ml./gal.*	*Ml./gal.*
1	4.00	4.00	4.00	3.99
2	4.00	4.00	4.00	4.00

Table V. Influence of Ethyl Ether and Acetone Washings on Accuracy of Results

	Tetraethyllead Present	Tetraethyllead Found Without acetone-ether wash	Tetraethyllead Found With acetone-ether wash
	Ml./gal.	*Ml./gal.*	*Ml./gal.*
	Gasoline C		
Determined as			
$PbSO_4$	2.99	3.02	2.99
$PbCrO_4$ (gravimetric)		3.03	3.00
$PbCrO_4$ (volumetric)		2.91	3.00
	Gasoline D		
$PbSO_4$	3.99	4.01	3.99
$PbCrO_4$ (gravimetric)		4.02	4.00
$PbCrO_4$ (volumetric)		3.96	3.99

Influence of Small Amounts of Copper

The small amount of copper added to the extraction solution serves as an indicator in neutralization. Analysis with known amounts of lead demonstrates the effect on accuracy. The results in Table IV show that the small amount of copper added to the extraction solution does not interfere with the determination of lead in either the volumetric or gravimetric methods.

Influence of Ether and Acetone Washings on Accuracy

The influence of acetone and ethyl ether washings on the accuracy of results was determined (Table V).

The acetone and ether washings not only speed up the test but are essential for accurate results both in the volumetric method and in the gravimetric methods when the lead is extracted from gasoline by the nitric acid–chlorate method. In the lead sulfate method, the acetone removes all traces of the lead acid solution; in the lead chromate method, washing with acetone removes all traces of organic matter that otherwise would tend to give high results when weighed and low results when titrated because of incomplete solubility of the lead chromate in the hydrochloric acid used. After the lead chromate has been washed with acetone and ether and the ether expelled, the resulting lead chromate is completely soluble in hydrochloric acid solution.

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Determination of Tin Coating Weights

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A procedure is described for the rapid determination of tin coating weights on tin plate. The tin is removed electrolytically in the presence of iodine and the excess iodine is back-titrated with sodium thiosulfate. The method is applicable to heavy and light coatings.

CHEMICAL analyses to determine the weight of tin coatings applied to sheet steel are made by producers and large consumers of tin plate. Since control laboratories performing this work may be called upon to make several thousand determinations per week, it is highly desirable that these determinations be made rapidly and economically. The necessity for performing a rather large number of tin coating determinations in this laboratory has instigated an investigation of the existing procedures.

The procedure most commonly used where a large number of determinations are to be made is that of Sellars (*4*). A definite area of tin plate, usually 4 square inches (25.8 sq. cm.) per side, is dissolved with heat in strong hydrochloric acid in an atmosphere of carbon dioxide. After cooling, stannous tin is titrated iodometrically to a starch end point with either standard iodine-iodide or iodate-iodide. This method is sufficiently accurate but fairly costly. Its speed is governed by the rate of solution of the steel samples and by the amount of equipment available for dissolving and cooling the samples under an inert atmosphere.

More recently Buser (*1*) has accomplished the solution of tin coatings electrolytically and without the aid of heat or the necessity for an inert atmosphere. In this method the tin plate specimen is made the anode between two carbon cathodes. The electrodes are immersed in a beaker containing hydrochloric acid, to which has been added a measured amount of standard iodate-iodide solution. Passage of a direct current through the cell for a few minutes results in solution of the tin in the stannous state Oxidation by iodine to the stannic state follows immediately. The excess of iodine is then titrated with sodium thiosulfate and the amount of tin is calculated from the decrease in iodine. Calculated stoichiometrically, high results will be obtained. Buser has, therefore, applied a correction factor obtained by comparison of his results with those obtained by other methods. Buser's method has definite advantages of speed and economy, but these are offset by the necessity for strict control of current density and of the length of time during which the cell is operated.

Several solutions, which have the ability to remove tin preferentially from the base metal, have been utilized for quantitative determinations of tin on tin plate. These are usually referred to as stripping solutions, and determinations based upon their use consist of weighing the sample before and after removal of the coating (*2*). Antimony trichloride in hydrochloric acid and alkaline lead acetate solutions have found favor in cases where laboratory facilities are limited. Stripping methods are comparatively time-consuming and do not quantitatively remove that portion of the tin which is alloyed with the iron.

Theoretical

When an electric current is passed through a cell consisting of carbon cathodes and a tin plate anode in a medium of iodine-

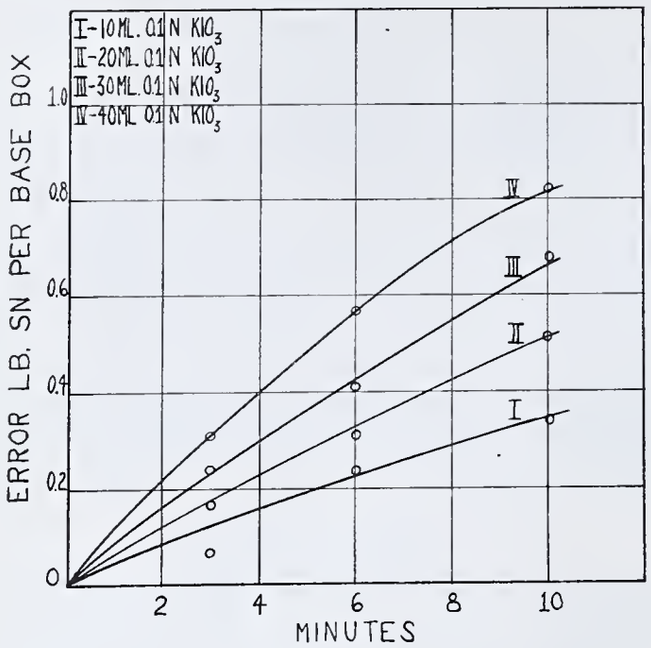

Figure 1. Effect of Iodine Concentration

iodide in hydrochloric acid, the following reactions occur, if there are no side reactions:

$$Sn = Sn^{++} + 2e$$
$$Sn^{++} + I_2 = Sn^{++++} + 2I^-$$
$$2H^+ + 2e = H_2$$

The amount of iodine consumed is therefore proportional to the amount of tin on the sample. In a cell of this type, as designed by Buser, there are several factors which may contribute errors, the sum of which is usually positive.

a. A negative error due to incomplete solution of tin under the clamp or clip which attaches the sample to the electrical circuit.

b. A negative error due to incomplete solution of tin or iron-tin alloy in cases where the current is not allowed to flow sufficiently long. (On tin plate made by immersing sheet steel in molten tin, a layer of iron-tin alloy, $FeSn_2$, is always present between the pure tin and the iron base metal. It may be dissolved anodically, *3*.)

c. A positive error due to the reduction of iodine by the ferrous iron generated at the anode.

d. A positive error due to the reduction of iodine by hydrogen generated at the cathode.

e. Negative errors which result from air-oxidation of iodide and stannous tin.

Error *a* is dependent upon the size of the clamp or clip used to attach the specimen and is constant if the area covered by the holder is the same for each sample specimen.

Under the conditions described by Buser, error *b* is negligible if the cell is operated for a sufficiently long time.

The magnitude of error *c* varies with the concentration of iodine in the cell and with the time the current is allowed to flow. This is shown in Figure 1 where the error, expressed as tin, is plotted against the time of cell operation for four different concentrations of iodine. (Tin coatings are expressed as pounds of tin per base box, one base box having an area of 31,360 square inches per side.)

To obtain the data for these curves, the tin was first stripped completely from a 4-square-inch tin plate specimen. The remaining base metal was then made the anode in a cell similar to that of Buser's, except that a porous wall was interposed between the cathode and anode chambers. Various amounts of 0.1 *N* iodide-iodate solution were introduced into the anode chamber together with 200 ml. of 1 to 4 hydrochloric acid. The cathode chamber contained only hydrochloric acid. A current of 3 amperes was allowed to flow through the cell for various time intervals and the decrease in iodine was determined by titrating with sodium thiosulfate. By confining the iodine to the anode chamber reactions occurring at the cathode were eliminated from consideration.

Figure 1 shows that the greater the concentration of iodine the greater will be the reduction of iodine. In an actual determination involving tin plate, relatively little iron is dissolved until after the tin has been removed. It is, therefore, unlikely that there is appreciable reduction of iodine by ferrous iron until after the tin has been stripped from the specimen.

Error *d* has been demonstrated experimentally by immersing a carbon cathode in a cathode chamber containing a measured amount of iodine-iodide in 1 to 4 hydrochloric acid, and separated from the anode chamber by a porous wall. After a current of 1.5 amperes had flowed through the cell for various time intervals, the loss of iodine was determined by titrating with thiosulfate. Under these conditions any decrease in iodine may be attributed to reactions occurring at the cathode. The curves in Figure 2 show that where the cell is operated at 1.5 amperes a loss of iodine equivalent to 0.05 pound of tin per base box occurs for each minute of cell operation.

Error *d* can be held at a minimum by allowing the current to flow only long enough to dissolve the tin. During an actual determination this cannot be done without danger of incomplete solution of tin from the sample (error *b*). Unlike error *c*, which does not occur until after the tin coating has been removed, error *d* occurs throughout the electrolysis if hydrogen is liberated in the presence of iodine.

Errors *e*, which are caused by dissolved oxygen, produce low results. The oxidation of iodide to iodine will be negligible if concentrations of iodide and acid are not excessive and the analysis is completed without undue delay. That the oxidation of stannous tin by oxygen will be prevented if iodine is present throughout the electrolysis, is demonstrated below.

Apparatus and Procedure

In order to eliminate error *d*, which is the most significant, a cell (Figure 3) was constructed. In this cell the cathodes are surrounded by porous cylinders (Coors filter, No. 720, size 1) which allow the hydrogen to escape without coming in contact

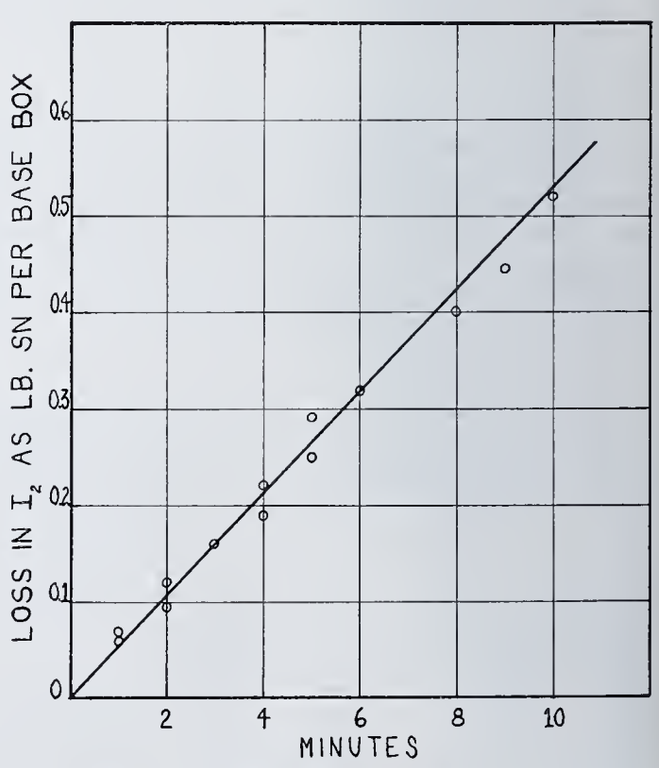

Figure 2. Hydrogen Reduction at Cathode

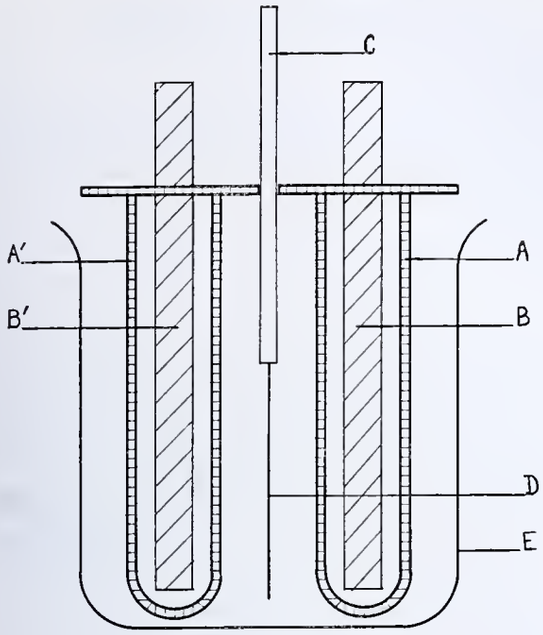

FIGURE 3. DETINNING CELL

with the iodine. A and A' are two identical porous filters filled with 1 to 4 hydrochloric acid and containing cylindrical carbon cathodes, B and B'. C is a small electromagnet (coil not shown) which holds the sample, D, at its edge without covering the tin surface, thus eliminating error a. E is a 400-ml. beaker into which the standard iodine is measured, together with 1 to 7 hydrochloric acid until the solution just covers the sample.

A source of direct current of from 3 to 5 volts will be sufficient to give about 3 amperes, through the cell shown in Figures 3 and 4. The current need not be controlled accurately to 3 amperes, but it has been found by experience that this amperage will remove the tin about as rapidly as titrations can be made. As a source of current an 8-volt storage battery, a direct current motor generator, or a dry rectifier transformer is satisfactory.

REAGENTS REQUIRED. *Hydrochloric Acid.* Dilute C. P. hydrochloric acid, with seven times its volume of distilled water. (Tap water may be used, providing it is free from objectionable oxidizing or reducing substances.)

Iodate-Iodide Solution. Dissolve 50 grams of potassium iodate, 280 grams of potassium iodide, and 0.1 gram of sodium hydroxide in 18 liters of distilled water.

Sodium Thiosulfate. Dissolve 365 grams of sodium thiosulfate pentahydrate in 18 liters of distilled water and add 2.5 grams of sodium carbonate.

Weaker solutions are preferable for light-weight coatings, 0.5 pound per base box or less.

PROCEDURE. Introduce 200 ml. of 1 to 7 hydrochloric acid and about 20 ml. of the iodate-iodide solution, accurately measured, into the 400-ml. beaker. Suspend the 4-square-inch tin plate sample from the magnet and immerse the electrodes in the beaker. Immersion of the electrodes in the beaker completes the electrical circuit. Allow a current of 3 amperes to flow for 2 to 5 minutes, remove the beaker, and titrate the remaining iodine with thiosulfate with starch as an indicator.

CALCULATIONS. The weight of tin coating for samples having an area of 4 square inches per side may be calculated stoichiometrically as follows:

$$(V_{KIO_3} \times N_{KIO_3} - V_{Na_2S_2O_3} \times N_{Na_2S_2O_3})\ 118/2 \times 17.28 = \text{pounds of tin per base box}$$

N = normality
V = volume in liters

Where a large number of determinations are to be made, it is convenient to construct a table or curve which gives directly the tin coating corresponding to various thiosulfate titrations.

Discussion of Results

EFFECT OF TIME OF ELECTROLYSIS. In Figure 5 the percentage of recovery of tin is plotted against the time of cell operation. To obtain the data for these curves the tin coatings on 4-square-inch disks were determined by the electrolytic stripping procedure. The Sellars method was used to determine the tin coating on the annular areas immediately surrounding the disks. Percentage recoveries were calculated on the assumption that the Sellars method gave the true tin coating.

The curves show that the tin is stripped rapidly. Following this, the curve becomes practically horizontal, although it slopes slightly upward, as a result of reduction of iodine by ferrous iron as predicted by Figure 1. A negligible error will be obtained if the cell is not operated for more than 4 to 5 minutes, which is greatly in excess of the stripping time conveniently used in making routine determinations. Because the slope of the top portion of the curve is dependent upon the concentration of iodine at the end of the determination, it is desirable not to use a large excess of iodine.

EFFECT OF ACID CONCENTRATION. The effect of acid concentration in the cell was investigated between concentrations 1 to 20 and 1 to 4. Below a concentration of 1 to 10 the stripping is incomplete and erratic results are obtained.

EFFECT OF AIR OXIDATION. The procedure as outlined could be simplified somewhat if stannous tin were stable toward air-oxidation, since it would then be possible to make the electrolysis without the presence of iodine and titrate it directly with standard iodate-iodide. To demonstrate the magnitude of air-oxidation, curves shown in Figure 6 were constructed. The data for these curves were obtained by the regular procedure, except that instead of having iodine present during the electrolysis, the solution was titrated with iodate-iodide to a starch end point after the electrolysis.

In curve II, Figure 6, boiled hydrochloric acid was used and the recoveries approach more nearly 100 per cent, since less oxygen was present. In a few other cases almost 100 per cent recovery was obtained by covering the boiled hydrochloric acid with a layer of toluene, thereby preventing solution of oxygen during the determination.

From these curves it is clear that unless an excess of iodine is maintained throughout the electrolysis low results will be obtained.

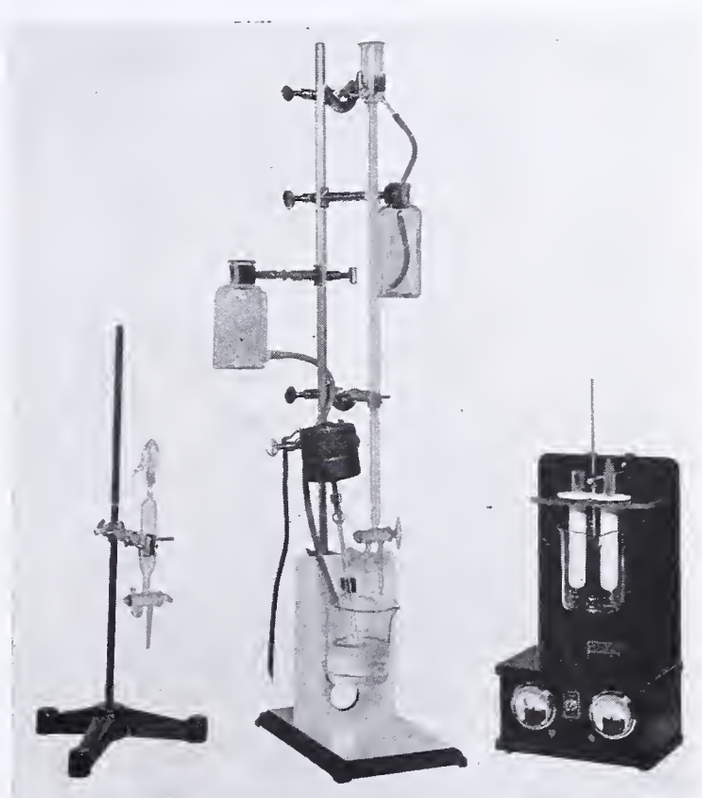

FIGURE 4. DETINNING CELL

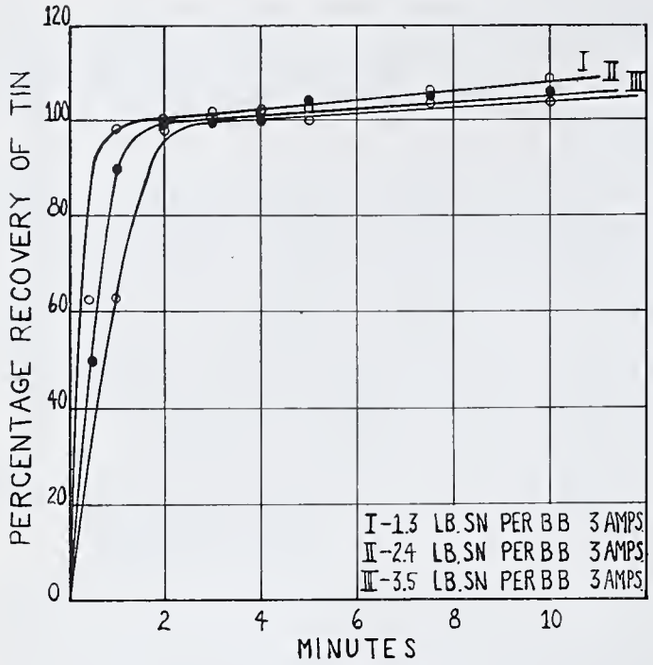

Figure 5. Recovery of Tin

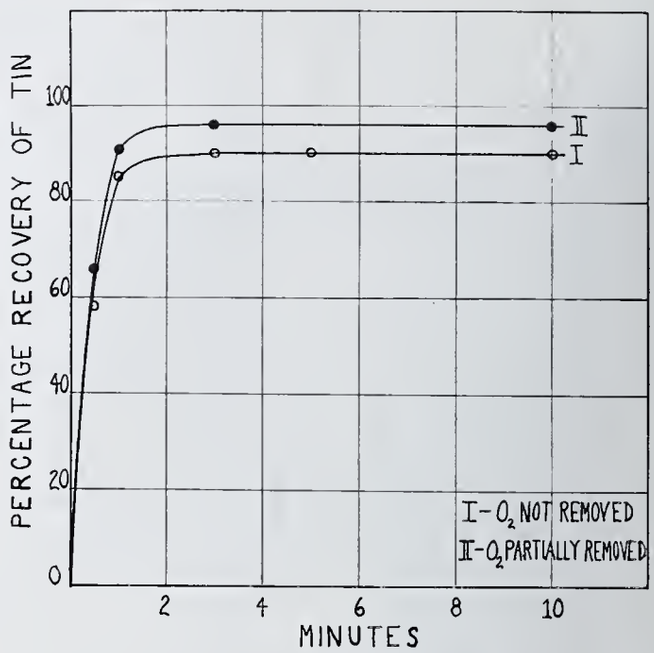

Figure 6. Air Oxidation

Accuracy. The accuracy of the procedure may be judged from the curves in Figure 5 and from Table I, in which the results obtained by the electrolytic stripping method are compared with those obtained by the Sellars method, as found by another laboratory. Four-square-inch disks were used for the electrolytic method and adjoining annuli for the Sellars method.

Since the introduction of electrodeposited tin coatings, considerably lighter tin coatings have been encountered. For such coatings (0.5 pound or less per base box) the electrolytic stripping determination is better than the Sellars method, which is always subject to errors resulting from iodine-consuming constituents of the base metal which are necessarily titrated with the tin. For this reason Sellars iodate solutions are standardized against pure tin to which have been added approximately 4 square inches of typical untinned base metal.

Table I. Comparison of Sellars and Electrolytic Methods

Sample	Electrolytic	Sellars	Difference
	Pounds of tin per base box		
1	2.02	2.02	0.00
2	2.03	2.03	0.00
3	2.10	2.10	0.00
4	1.58	1.58	0.00
5	1.95	1.94	+0.01
6	1.48	1.48	0.00
7	1.70	1.70	0.00
8	1.52	1.54	−0.02
9	1.99	2.01	−0.02
10	2.07	2.03	+0.04
11	1.83	1.85	−0.02
12	1.42	1.33	+0.09
13	2.15	2.13	+0.02
14	1.50	1.53	−0.03
15	1.59	1.61	−0.02

Table II. Comparison of Methods

Sample	Sellars	$SbCl_3$ Stripping	$SbCl_3$ Stripping Corrected	Plumbite Stripping	Electrolytic
	Pounds of tin per base box				
1	0.43	0.46	0.44	..	0.43
	0.43	0.46	0.45	..	0.45
	0.42	..	..	..	0.44
2	0.43	0.45	0.45	0.43	0.42
	0.42	0.46	0.45	0.44	0.42
3	0.43	0.45	0.44	0.43	0.43
	0.43	0.44	0.44	0.43	0.42
4	0.45	0.46	0.45	0.44	0.43
	0.44	0.47	0.45	0.45	0.44

This procedure is entirely adequate when the amount of iodine required by the base metal is constant and small compared with that consumed by the tin; but when light-weight coatings are determined the Sellars method is accompanied by an appreciable error if the iodine-consuming properties of the base metal are not accurately taken into account.

Table II shows a comparison of the Sellars, electrolytic stripping, and chemical stripping methods.

In the case of the Sellars method the iodate solution was standardized against pure tin, to which had been added 4 square inches of the same base metal found in the samples. In the electrolytic stripping method the strength of the titrating solutions was reduced to one fifth that of the regular procedure. The stripping solutions consisted of 1 per cent antimony trichloride in 20 per cent hydrochloric acid in one procedure and a solution 5 per cent with respect to lead acetate and sodium hydroxide in another. In the case of the acid stripping solution, the results have been corrected for the loss in weight due to solution of iron during the stripping. No such correction was necessary in the case of the alkaline stripping solution. Samples for data shown in Table II consisted of strips of commercial electrodeposited tin plate. These were taken in a direction parallel to passage through the plating tank, so that the tin coatings of specimens taken for the various methods should be essentially constant.

The results given in Table II show satisfactory agreement between the various methods, although antimony trichloride stripping gives slightly high results. The agreement between the Sellars and electrolytic stripping would not be expected to be so good if the iodine-consuming properties of the base metal had not been accurately taken into consideration.

Acknowledgment

The authors wish to express their appreciation to Mr. W. C. Burfisher of the Wilkens-Anderson Company for assistance in designing the stripping unit shown in Figure 4. (This equipment is offered for sale by the Wilkens-Anderson Company, 111 North Canal St., Chicago, Ill.)

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Determination of Metallic Copper and Cuprous Oxide

In Commercial Cuprous Oxide Pigments

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Cuprous oxide is readily soluble in cold, dilute aqueous hydrochloric acid, cupric oxide is slightly soluble, and metallic copper is very slightly soluble. The presence of hydrazine sulfate reduces any divalent copper entering solution and prevents reaction between metallic copper and cupric ion. The addition of potassium chloride prevents the precipitation of cuprous chloride from the very dilute hydrochloric acid solution. If the analysis is performed under an inert carbon dioxide atmosphere the metallic copper and cupric oxide may be filtered off, dissolved in acid ferric chloride, and titrated with ceric sulfate. The total reducing power, the sum of the cuprous oxide and copper reducing equivalents, may be determined by direct solution of the sample in acid ferric chloride and subsequent titration under a carbon dioxide atmosphere with ceric sulfate.

COMMERCIAL cuprous oxide pigments contain in addition to cuprous oxide various percentages of metallic copper and cupric oxide. Because of its finely divided state, the metallic copper is soluble or partially soluble in the reagents that dissolve cuprous oxide, rendering its separation difficult. Considerable work has been performed to determine a method of separation of metallic copper–cuprous oxide mixtures.

Determination of Metallic Copper

LeBlanc and Sachse (*3*) determined the percentage of cupric oxide by titrating the iodine liberated by the oxidation of hydrochloric acid solutions of potassium iodide. Rapid oxidation of the copper by the liberated iodine eliminated this method as an analytical procedure. Fitzpatrick (*4*) attempted to separate the metallic copper from the mixture by boiling the sample with a saturated silver sulfate solution. During the 10-minute boiling extraction period a considerable amount of the cuprous oxide reacts to form metallic copper which is subsequently dissolved, giving high copper values. Zerfass and Willard (*6*) attempted to analyze the mixture microscopically. Their results were determined on mixtures containing approximately 50 per cent cupric oxide, whereas commercial cuprous oxide contains only a small per cent. The method was not found to be practical in its application to cuprous oxide pigments.

The use of reagents which dissolve cuprous oxide, cupric oxide, or both but have no effect on metallic copper presents many difficulties. Solution of the sample in the reagents invokes a reaction between cupric ions and copper, forming cuprous ion, particularly when the copper is in the finely divided state, as in commercial cuprous oxide pigments. To decrease the solubility of metallic copper, reducing agents were added which could reduce cupric ions to the cuprous state but not to metallic copper and would not affect the metallic copper. A few such reducing agents are hydrazine sulfate, hydroxylamine hydrochloride, and sodium arsenite.

Hurd and Clark (*1, 2*) outlined a method for the determination of metallic copper and cuprous oxide.

Cuprous oxide is soluble in cold aqueous ammonia, whereas cupric oxide is but slightly soluble and metallic copper is insoluble. Addition of hydrazine sulfate to the reagent tends to reduce any divalent copper entering solution and prevents interaction of cupric copper and metal. If the reaction be carried out in a carbon dioxide atmosphere, the metallic copper–cupric oxide mixture may be isolated and the metal determined by direct solution in acid ferric chloride, followed by titration with potassium dichromate.

The precautions required in this method are numerous and are difficult to apply. An extensive investigation of this method was carried out, and the results obtained by slight variations in procedure are outlined in Table I. Difficulties encountered are as follows:

1. The method depends on the ability of the analyst to determine when all the cuprous oxide has dissolved, by observation of the disappearance of red particles from a mixture of brownish red copper and black cupric oxide in a blue solution. Moreover, cuprous oxide may exist in a variety of colors ranging from yellow through red to brown, depending on the particle size.
2. It is difficult to control the solubility of carbon dioxide in the alkaline extracting solution. A change in rate of introducing carbon dioxide gas over the solution markedly affects the solubility of the cuprous oxide. The pressure over the solution also affects the solubility. Carbon dioxide is a necessary ingredient of the extraction solution and acts as an active solvent, owing to the formation of ammonium carbonate. Therefore, variations in the quantity of carbon dioxide taken up by the solution during extraction alter the results. Replacing carbon dioxide with other inert gases results in very high copper values.
3. The presence of a thin film of cupric oxide due to oxidation prevents solution of all cuprous oxide unless vigorous agitation is applied. This results in lower copper values due to the increased solubility of the copper under agitation.
4. The method involves a large personal factor which makes duplicable results difficult to obtain. Results obtained by different operators failed to check.
5. The time of extraction markedly affects the results.

Various solvents, reducing agents, and procedures were investigated by this laboratory to find a method that would quantitatively separate the copper in commercial cuprous oxides and eliminate the personal factor of the Hurd and Clark method (*1, 2*). Various solutions of hydrochloric

TABLE I. METALLIC COPPER BY HURD AND CLARK METHOD (*2*)

Sample	Cu, %	Conditions of Experiment
Powder Metals and Alloys, 76	8.73	Extracted 5 min.
	7.01	Extracted 5 min.
	4.13	Extracted 30 min.
	20.4	No CO_2, open to air, extracted 30 min.
	23.1	H_2 atmosphere, extracted 25 min.
	15.78	H_2 atmosphere, extracted 10 min.
A. S. T. M., 1	4.92	(Extracted to disappearance of red particles)
	6.49	(Extracted to disappearance of red particles)
	13.69	(Very little agitation, slow stream of CO_2)
Powder Metals and Alloys, 79	14.76	Extracted 3 min.
	12.93	Extracted 5 min.
	6.86	Extracted 10 min.
	5.55	Extracted 15 min.
	4.04	Extracted 30 min.
A. S. T. M., 4	2.48	(Extracted to disappearance of red particles)
	3.88	(Extracted to disappearance of red particles)
	9.19	(Extracted to disappearance of red particles)
	2.67	(Extracted to disappearance of red particles)
	7.2	(Extracted to disappearance of red particles)

acid and ammonium hydroxide with such reducing agents as hydroxylamine hydrochloride, mercury, formaldehyde, sugars, sodium arsenite, hydrazine sulfate, and others were tested. None of these methods gave reproducible results for the determination of metallic copper.

A series of experiments was conducted, using varying concentrations of hydrochloric acid in conjunction with hydrazine sulfate. When the hydrochloric acid concentration dropped below a certain minimum, the cuprous chloride formed from the reaction precipitated. To remove this precipitate from the copper residue potassium chloride was added to the extraction mixture. The result was that a very low hydrochloric acid concentration could be employed which was still sufficient to dissolve the cuprous oxide but insufficient to react with the copper, despite the fact that it is finely divided. The hydrazine sulfate was added to prevent interaction of copper and cupric ion. Carbon dioxide had no effect on the solubility of the cuprous oxide in the extracting solution.

Reagents Required. *Ferric Chloride Solution.* Add 75 grams of ferric chloride hexahydrate and 150 ml. of hydrochloric acid (specific gravity 1.19) to 400 ml. of distilled water saturated with carbon dioxide. Stir to complete solution. Keep the solution saturated with carbon dioxide.

Hydrazine Sulfate Solution (3 per cent). Add 3 grams of pure hydrazine sulfate to 97 ml. of distilled water saturated with carbon dioxide. Stir to complete solution.

Potassium Chloride Solution (10 per cent). Dissolve 10 grams of c. p. potassium chloride in 90 ml. of distilled water saturated with carbon dioxide.

Acetone, chemically pure reagent grade.

Indicator, o-phenanthroline ferrous complex (ferroin), manufactured by G. Frederick Smith Company, Columbus, Ohio.

Ceric Sulfate Solution. Weigh 50 grams of anhydrous ceric sulfate into an 800-ml. beaker and add 26 ml. of concentrated sulfuric acid. Dilute with 30 ml. of water. Heat several hours with occasional stirring, replacing water lost by evaporation. Add water in small portions at half-hour intervals until the volume reaches about 700 ml. Cool, transfer to a 1-liter volumetric flask, and dilute to mark. Allow solution to settle several weeks and filter to remove suspended matter. Standardize against electrolytic iron or ferrous ammonium sulfate.

Ferrous Ammonium Sulfate. Dissolve 12 grams of Mohr's salt in 200 to 300 ml. of water and add 40 ml. of concentrated sulfuric acid, stirring constantly. Dilute to 1 liter in a volumetric flask. Add a few pieces of mossy aluminum to stabilize the solution. The solution should be restandardized frequently with 0.1 *N* potassium dichromate or ceric sulfate.

Carbon Dioxide–Saturated Distilled Water. Boil distilled water for several minutes and pass a rapid stream of carbon dioxide gas through the solution while cooling. Keep water saturated with carbon dioxide gas.

Procedure. Add 10 ml. of c. p. acetone and a few small glass beads to a 250-ml. glass-stoppered, Squibb's type separatory funnel. Replace air by passing carbon dioxide gas into funnel for a few minutes or adding a lump of carbon dioxide snow. Weigh accurately 1 gram of the well-mixed sample and transfer directly to the separatory funnel. Shake vigorously for 1 minute to dissolve any oil or surface coatings and to disperse and wet the particles thoroughly. Refill funnel with carbon dioxide gas, add 20 ml. of dilute hydrochloric acid solution (1 to 9) and 10 ml. of hydrazine sulfate solution (3 per cent), and shake gently for 1 minute. Remove stopper from funnel, refill with carbon dioxide gas, and add 75 ml. of 10 per cent potassium chloride solution. Shake vigorously for 15 seconds. Filter off the metallic copper–cupric oxide residue in the usual way through a 12.5-cm. 41-H Whatman filter paper and a glass funnel. Allow all of the solution to drain off completely before washing. Wash once with 30 ml. of 10 per cent potassium chloride solution and once with 30 ml. of 5 per cent potassium chloride solution. Keep an atmosphere of carbon dioxide over the solution during filtration by adding small pieces of carbon dioxide snow. Finally, wash with approximately 400 ml. of distilled water saturated with carbon dioxide, with the further addition of small pieces of carbon dioxide snow during the washing. Place the washed residue and filter paper in a wide-mouthed 250-ml. Erlenmeyer flask from which the air has been replaced by carbon dioxide gas. Add 15 ml. of the ferric chloride solution and 15 ml. of (1 to 9) hydrochloric acid solution. Warm gently on a steam bath to dissolve the metallic copper–cupric oxide residue, still maintaining a carbon dioxide atmosphere in the flask. Cool the solution to room temperature in carbon dioxide atmosphere. Add 2 drops of *o*-phenanthroline (ferrous complex) indicator and titrate at once with 0.1 *N* ceric sulfate solution. A sharp, distinct color change from orange to pale green occurs at the end point. Back-titrate with 0.03 *N* ferrous ammonium sulfate to an orange color. Subtract the ferrous ammonium sulfate equivalent from the total titration and calculate the percentage of metallic copper and report as such.

1 ml. of 0.1 *N* ceric sulfate = 0.003179 gram of copper.

Precautions. The analysis must be run entirely in an atmosphere of carbon dioxide with the exclusion of all air to prevent oxidation of copper. Carbon dioxide snow or dry ice has been found a convenient method of replacing air.

The sample must be well mixed to obtain a homogeneous mixture; otherwise checks cannot be obtained.

The solution containing the metallic copper–cupric oxide residue must be allowed to drain completely through the filter paper, before the final wash with water, to prevent precipitation of cuprous chloride on the filter paper.

Table II. Metallic Copper in Commercial Cuprous Oxide Samples

Hydrazine Sulfate–Potassium Chloride Method

	A. S. T. M., 6, Electrolytic		Rohm & Haas, Electrolytic		Metals Refining Co., C-18		Metals Refining Co., 41		Powder Metals and Alloys, 320		Powder Metals and Alloys, 99	
	Cu	Deviation from av.	Cu	Deviation from av.	Cu	Deviation from av.	Cu	Deviation from av.	Cu	Deviation from av.	Cu	Deviation from av.
	%	%	%	%	%	%	%	%	%	%	%	%
	0.09	−0.005	0.32	+0.05	0.61	−0.06	0.97	0.00	4.25	−0.05	6.5	+0.17
	0.09	−0.005	0.22	−0.05	0.66	−0.01	0.99	+0.02	4.28	−0.02	6.25	−0.08
	0.10	+0.005	0.27	0.00	0.73	+0.07	0.94	−0.03	4.34	+0.04	6.39	+0.06
	0.10	+0.005					0.96	−0.10	4.32	+0.02	6.29	−0.04
											6.39	+0.06
											6.42	+0.09
											6.23	−0.10
Av.	0.095	0.005	0.27	0.03	0.67	0.05	0.97	0.02	4.30	0.03	6.33	0.09

	Powder Metals and Alloys, 100		Metals Refining Co., C-16		Powder Metals and Alloys, 113		Powder Metals and Alloys, 111		Powder Metals and Alloys, 105	
	Cu	Deviation from av.	Cu	Deviation from av.	Cu	Deviation from av.	Cu	Deviation from av.	Cu	Deviation from av.
	%	%	%	%	%	%	%	%	%	%
	6.58	+0.03	7.36	+0.01	7.68	+0.12	11.74	−0.02	12.56	−0.09
	6.44	−0.11	7.30	−0.05	7.55	−0.01	11.96	+0.20	12.69	+0.04
	6.52	−0.03	7.29	−0.06	7.50	−0.06	11.6	−0.16	12.71	+0.06
	6.63	+0.08	7.34	−0.01	7.44	−0.12	11.74	−0.02	12.63	−0.02
	6.57	+0.02	7.36	+0.01	7.53	−0.03			12.57	−0.08
			7.33	−0.02	7.52	−0.04			12.72	+0.07
			7.37	+0.02	7.67	+0.11			12.77	+0.12
			7.38	+0.03						
			7.36	+0.01						
			7.35	0.00						
			7.27	−0.08						
			7.47	+0.12						
Av.	6.55	0.05	7.35	0.035	7.56	0.07	11.76	0.10	12.65	0.06

Result of Analyses. This method of analysis was used for the determination of metallic copper in eleven samples of commercial cuprous oxides and a number of synthetic mixtures. A total of 79 determinations was run, and the results are outlined in Tables II and III. The method is direct and, with the precautions required, easily carried out. The personal factor required in the Hurd and Clark method (*1*, *2*) for the determination of loss of color, rate of carbon dioxide addition, etc., has been eliminated. With a little prac-

Table III. Metallic Copper in Synthetic Mixtures[a]

Hydrazine Sulfate–Potassium Chloride Method

2% Cu		3.65% Cu		6.02% Cu		8.1% Cu		14.2% Cu	
Found	Deviation from 2% Cu	Found	Deviation from 3.65% Cu	Found	Deviation from 6.02% Cu	Found	Deviation from 8.1% Cu	Found	Deviation from 14.20% Cu
%	%	%	%	%	%	%	%	%	%
1.98	−0.02	3.63	−0.02	5.92	−0.10	8.02	−0.08	14.20	0.00
1.96	−0.04	3.60	−0.05	5.97	−0.05	8.17	+0.07	14.45	+0.25
2.02	+0.02	3.67	−0.02	5.94	−0.06	8.21	+0.11	14.46	+0.26
1.96	−0.04	3.61	−0.04	5.98	−0.04	8.05	−0.05	14.10	−0.10
Av. 1.98	0.03	3.63	0.03	5.95	0.06	8.12	0.08	14.31	0.15

[a] Synthetic mixtures were prepared from Rohm & Haas electrolytic grade cuprous oxide C and 325-mesh metallic copper powder which was extracted with hydrochloric acid–hydrazine sulfate solution, washed, and dried in a vacuum desiccator. Results were corrected for small amount of metallic copper found in analysis of cuprous oxide.

tice the method will give results reproducible within the limits shown in Tables II and III. A maximum deviation of 0.1 per cent and an average deviation of 0.05 per cent was obtained with the electrolytic or low copper content cuprous oxides; and a maximum deviation of 0.3 per cent and an average deviation of 0.15 per cent with cuprous oxides of high copper content. Results determined on the same sample at different periods of time must not be compared for accuracy, since many samples of cuprous oxide change considerably from day to day.

Table IV. Total Reducing Power Using Potassium Dichromate

Metals Refining Co., 1011	Powder Metals and Alloys, 111	Powder Metals and Alloys, 99	Powder Metals and Alloys, 105
%	%	%	%
101.8	106.1	98.2	108.0
102.1	107.0	99.3	109.5
103.4	106.4	98.4	108.5
102.8			109.2

Determination of Total Reducing Power

Determining total reducing power of cuprous oxide pigments by solution of the sample in hydrochloric acid–ferric chloride solution, followed by titration of the resulting ferrous iron with potassium dichromate, using barium diphenylamine sulfonate indicator, was found to be unsatisfactory. Results obtained differed in some cases by as much as 1.5 per cent, although the procedure was identical in each case (*5*). Table IV lists the values obtained on a number of commercial samples.

In a report made by the National Bureau of Standards in March, 1942, on the analysis of samples of cuprous oxide, a value of 98.0 per cent was obtained for total reducing power on sample C-16 and 10 days later a value of 99.0 per cent was obtained. As samples of cuprous oxide lose reducing power during storage, this is not consistent with other results obtained on similar samples, which showed a loss in reducing power. The results obtained by two operators on samples C-17, C-18, and C-19 varied as much as 0.5 per cent when run at the same time, which is considered too large an error for the determination of total reducing power.

Numerous instances were found in determining the percentage of cupric oxide in the material by difference, in which the figure for total copper, calculated from the cuprous oxide and metallic copper results, was higher than the actual total copper determined electrolytically. Since it was possible to check the figure for metallic copper accurately, it appears that the error lies in the fact that the value for total reducing power was too high.

An investigation was undertaken to obtain an accurate reproducible method for the determination of the total reducing power of cuprous oxide pigments. Various methods of procedure, oxidizing titrating solutions, indicators, and acids were used in the investigation. It was found that varying the quantity of indicator varied the results, whereas the blank remained the same. Variation in the acids used slightly altered the results. The use of ceric sulfate solution as an oxidizing titrating solution produced accurate results.

The reagents required are the same as for the determination of metallic copper.

Procedure. Weigh accurately a 0.2-gram sample and place it in a 250-ml. vented, glass-stoppered Erlenmeyer flask previously filled with carbon dioxide gas. Add a few small glass beads and 10 ml. of ferric chloride solution. Heat gently for 15 minutes, stirring occasionally and maintaining a carbon dioxide atmosphere at all times. After the sample has dissolved, cool and titrate at once with 0.1 *N* ceric sulfate solution by the procedure given for the determination of metallic copper. Calculate the total reducing power as cuprous oxide and report as such.

1 ml. of 0.01 *N* ceric sulfate = 0.007157 gram of cuprous oxide.

To determine the percentage of cuprous oxide multiply the per cent of metallic copper by 2.252 and subtract from the per cent of total reducing power as cuprous oxide. The difference is percentage of cuprous oxide.

To determine percentage of cupric oxide, use the following formula:

$a = Cu_2O \times 0.888$ = metallic copper equivalent of Cu_2O
b = metallic copper as determined
c = total copper determined electrolytically
$c - (a + b) = d$ = per cent of copper as CuO
$d \times 1.252$ = per cent of CuO

Ceric sulfate, in the determination of total reducing power and metallic copper, has a number of advantages over potassium dichromate. In the case of the dichromate with diphenylamine sulfonate as indicator, a considerable quantity of indicator is necessary to give a visible color change at the end point. Often the change from deep green to purple is difficult to obtain sharply, even though an excess of phosphoric and sulfuric acid is present. With *o*-phenanthroline, 2 drops of indicator give a sharp, distinct color change at the

Table V. Analyses of Commercial Cuprous Oxide Pigments

Sample	Rohm & Haas C		Rohm & Haas F		Metals Refining Co., 113		Metals Refining Co., 1011	
	%	%	%	%	%	%	%	%
Metallic Cu	0.33	0.33	0.83	0.83	1.4	1.4	3.6	3.6
Total Cu (electrolytic)	86.07	86.07	86.70	86.70	88.10	88.10	88.01	88.01
Total reducing power: potassium dichromate	97.80	...	98.92	...	100.80	...	103.5	....
Total reducing power: ceric sulfate	...	97.00	...	98.20	....	99.70	....	102.1
Cu_2O (calcd.)	97.15	96.34	97.05	96.50	97.65	96.55	95.40	94.00
Total Cu calcd. from metallic Cu and Cu_2O	86.60	85.87	87.03	86.25	88.14	87.15	88.34	87.10
CuO, by difference	[a]	0.25	[a]	0.38	[a]	1.20	[a]	1.13

[a] Negative figure.

Table VI. Total Reducing Power Using Ceric Sulfate

	Metals Refining Co., 228		Metals Refining Co., 41		Powder Metals and Alloys, 320		Powder Metals and Alloys, 278	
	%	Deviation from av. %	%	Deviation from av. %	%	Deviation from av. %	%	Deviation from av. %
	99.31	+0.01	100.14	+0.06	99.03	−0.04	97.55	0.00
	99.36	+0.06	100.10	+0.02	99.09	+0.02	97.60	+0.05
	99.28	−0.02	100.05	−0.03	99.07	0.00	97.61	+0.06
	99.25	−0.05	100.03	−0.05	99.10	+0.03	97.49	−0.06
	99.35	+0.05	100.11	+0.03	99.07	0.00	97.53	−0.02
	99.23	−0.07	100.05	−0.03	99.04	−0.03	97.50	−0.05
Av.	99.30	0.04	100.08	0.04	99.07	0.02	97.55	0.04

Table VII. Total Reducing Power of Synthetic Mixtures of Cuprous Oxide and Cupric Oxide

Calculated	Dichromate Found	Dichromate Error	Ceric Sulfate Found	Ceric Sulfate Error
%	%	%	%	%
91.53	91.96	+0.43	...	....
92.71	...	....	92.61	−0.10
85.45	86.36	+0.91	...	....
83.28	...	....	83.40	+0.12
79.75	80.21	+0.46	...	....
77.68	...	....	77.60	−0.08
65.59	66.24	+0.65	...	....
66.32	...	....	66.16	−0.16
61.25	61.65	+0.40	...	....
60.08	...	....	60.11	−0.03

Synthetic mixtures were prepared by weighing varying amounts of cuprous oxide and cupric oxide to total approximately 0.25 gram and determining the total reducing power on the entire sample. Cuprous oxide was Rohm & Haas electrolytic grade cuprous oxide C (Table V). Cupric oxide was a reagent grade material with less than 0.1 per cent total reducing power and screened through a 325-mesh screen.

end point with ceric sulfate. No additional acids are required. This indicator cannot be used with dichromate, since the color change from orange to green is obscured by the orange color of the dichromate ion. The cerous ion on the other hand is colorless. Ceric sulfate is applicable in the determination of reducing agents in the presence of high concentrations of hydrochloric acid. The oxidation reduction potential of the ceric ion is very high, being close to that of permanganate, thus ensuring a rapid, complete reaction.

Table V gives analyses of four commercial cuprous oxides, using the dichromate and ceric sulfate methods for determining total reducing power. In the case of the dichromate titration, the copper calculated from metallic copper and cuprous oxide is more than is found by determining total copper electrolytically. The ceric sulfate method shows sufficient copper to allow for the presence of cupric oxide. This is more in accordance with what is to be expected, since it is doubtful that commercial cuprous oxides can be manufactured with the total exclusion of cupric oxide.

Table VI lists the results of 24 determinations of four commercial cuprous oxide samples for total reducing power by the ceric sulfate method. A maximum deviation of 0.1 per cent and an average deviation of 0.04 per cent were obtained. Table VII shows the results obtained for synthetic cuprous-cupric oxide mixtures by the dichromate and ceric sulfate methods.

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The views presented in this article are those of the writers and are not to be construed as the official views of the Navy Department.

Determination of Butadiene

In the Presence of Other Unsaturated and Saturated Gaseous Hydrocarbons

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A RAPID and accurate method of analysis for 1,3-butadiene is of considerable importance in the present synthetic rubber program. It is well known that the various methods of preparation of butadiene give products consisting of mixtures of compounds, some of which are of a particularly complex character. Depending upon the method of manufacture butadiene can be associated with any of the following hydrocarbons: *n*-butane, isobutane, isobutene, 1-butene, *cis*-2-butene, *trans*-2-butene, methylacetylene, vinylacetylene, and ethylacetylene. The method of analysis for butadiene in gaseous mixtures should be rapid and accurate, regardless of the percentage of the above-mentioned compounds.

At the present time the usual method of analysis for butadiene is based on its reaction with molten maleic anhydride at 100° C. (*2*, *6*). When butadiene exists in the presence of other gases, such as isobutene, butenes, and butanes, a single absorption cannot be used, since the gases associated with the butadiene will be absorbed by the maleic anhydride, giving incorrect results. To overcome this error the molten maleic anhydride must first be treated with a portion of the sample to be tested so that equilibrium with all gases present exclusive of butadiene, particularly isobutene, will be established. When equilibrium is reached, another portion of the gaseous mixture is again absorbed, and the operations are repeated until check values for the butadiene are obtained.

It is desirable to estimate first the amount of butadiene in the gaseous mixture before carrying out the determination, since it has been found necessary by some investigators to dilute the mixture with nitrogen when the butadiene is present in high percentages.

This paper describes a method for the determination of butadiene in gaseous hydrocarbon mixtures free of acetylenes. It has been found possible to remove the acetylenes commonly associated with the butadiene and butenes pro-

duced by cracking petroleum stocks without affecting the relative proportion of the remaining gaseous constituents. Details of this method will be published later.

The determination of butadiene consists of two fundamental operations: (1) absorption of the alkenes and alkadienes in a mercuric nitrate solution; (2) hydrogenation of the alkenes and alkadienes to alkanes.

From these data the mole per cent of alkadienes in the hydrocarbon mixture may be calculated by the following equation:

mole % alkadiene = (mole % unsaturation by hydrogenation) − (mole % unsaturation by mercuric nitrate absorption)

Apparatus and Material

All inorganic chemicals used were of reagent grade. The anhydride was obtained from the Eastman Kodak Company. The apparatus required for the analysis of hydrocarbon gases containing butadiene consists of the following:

1. Any suitable type of analytical hydrogenation apparatus can be used. For this work the apparatus was patterned after the design described in the Universal Oil Products handbook with no essential modification (*7*).
2. An Orsat apparatus, having the gas buret calibrated so that the smallest division is not greater than 0.1 ml. and equipped with at least three bubblers, preferably the Fisher valve bubbler-type pipet (*4*) is used.
3. The solutions used in the bubbler pipets are 30 per cent potassium hydroxide in one and mercuric nitrate solution in the other two. The second pipet, containing mercuric nitrate solution, is used alternately with the first one.

The most satisfactory composition of the mercuric nitrate reagent is:

600 grams of mercuric nitrate, $Hg(NO_3)_2$
1250 grams of sodium nitrate, $NaNO_3$
383 ml. of 70 per cent nitric acid, HNO_3
2100 ml. of distilled water

Procedure

The sample is passed through mercuric nitrate solution until the absorption is complete. This operation requires five or six cycles if a fresh solution is in the bubbler at the beginning of the operation. When the solution is partially spent, additional passes may be required to obtain the same result. Samples rich in isobutene will cause a yellow precipitate to form, which at first is redissolved by the reagent. Further additions of isobutene cause a permanent precipitate to form and collect on the surface of the pipet. At this point it is necessary to replace the solution with fresh mercuric nitrate reagent. After complete absorption in the mercuric nitrate pipet, the remaining gas is scrubbed in the caustic bubbler to remove any traces of nitric acid. Both the alkenes and alkadienes are absorbed by the mercuric salt. If the gas is completely or nearly soluble in the solution, it is necessary to add some inert gas to act as a carrier to enable the gas to contact the reagent effectively; air may be used for this.

The hydrogenation value of an aliquot portion of the same gas sample is now determined by mixing a known volume of the unknown gas with an excess amount of oxygen-free hydrogen (*3*). Since the catalyst is poisoned by oxygen, carbon monoxide, or hydrogen sulfide, it is essential that the gas be free of these constituents. The presence of moisture will also lead to erroneous results, so the gas sample and the hydrogen should be thoroughly dried before they are introduced into the measuring burets. The resulting mixture is then passed through the nickel catalyst until a constant volume is reached. The decrease in volume is calculated in terms of percentage hydrogenation after applying a correction for the deviation of the hydrogen-hydrocarbon mixture from the ideal gas volume (*1*, *5*).

Experimental Results

The method was applied to synthetic blends of *n*-butane, isobutene, and butadiene. These known mixtures were prepared by weighing definite amounts of each hydrocarbon gas into a stainless steel bomb. A comparison of the butadiene content by the maleic anhydride method for known blends is given in Table I.

Table I. Butadiene Content of Synthetic Blends

No.	Blend n-C_4H_{10}	Blend i-C_4H_8	Blend C_4H_6	Unsaturation By hydrogenation	Unsaturation By $Hg(NO_3)_2$ solution	C_4H_6 Determined By hydrogenation	C_4H_6 Determined By $Hg(NO_3)_2$	C_4H_6 Determined By maleic anhydride
	%	%	%	%	%	%	%	%
1	16.3	0	83.7	167.4	83.7	83.7		83.6
2	15.5	19.3	65.2	149.9	84.7	65.2		64.0
3	17.8	29.9	52.3	134.6	82.4	52.2		51.8
4	21.6	45.6	32.8	113.6	81.1	32.5		31.8
5	18.2	53.4	28.4	110.1	81.7	28.4		28.6
6	19.6	65.1	15.3	95.7	80.6	15.1		15.0

Discussion

The results obtained by maleic anhydride absorption, though fairly close in most cases, fail to agree with the known values as well as the results obtained by this method. In carrying out the maleic anhydride method the pressure above the molten anhydride must be kept as close to atmospheric as possible. Substantial variations in the pressure cause a considerable difference in the amount of nonreactive gases which will dissolve in maleic anhydride. It must also be assumed that the solubility of the gases, other than the butadiene in the tetrahydrophthalic anhydride, is the same as it is in the maleic anhydride.

It has been observed that, before an operator can obtain reproducible results by the maleic anhydride method, a certain amount of manipulative skill must be acquired, whereas the method described requires little experience to give satisfactory results.

Isobutene was used in the blends (see Table I) in place of some other butene, because there has been some question as to whether the mercuric nitrate unsaturation value could be depended upon when the sample contained isobutene. The tabulated results show that quantitative absorption of isobutene, as well as butadiene, can be easily accomplished, provided the mercuric nitrate reagent is replaced as soon as any appreciable crystallization of the hydrocarbon-mercuric salt occurs in the solution.

The recovery of mercury from the spent solutions is accomplished by collecting the spent reagent in a jar and suspending a strip of lead in the liquid. The solution should be made acid with concentrated nitric acid. Lead will replace the mercury in solution, and metallic mercury will collect in the bottom of the jar. This helps to eliminate the cost involved by the use of mercury salts.

Acknowledgment

The authors wish to thank R. S. Crog for suggesting the extension of the hydrogenation method to include butadiene, and Margaret Jean Saltsman for her assistance.

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Determination of Iron in Water

A Modified Colorimetric Procedure

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THE method described was developed because of the need in routine water analysis for a more satisfactory and less time-consuming procedure than that in the standard methods (*1*). The presence of iron is of concern in the treatment, distribution, and use of water, particularly iron as soluble salts or as suspended oxides in "red waters". Iron in either form is readily soluble in hot acid solutions. Insoluble iron compounds, such as are present in suspended soil particles, are of relatively minor significance in water conditioning; they do not indicate corrosion, nor is the deleterious effect of soil suspensions due primarily to their iron content. The basic features of the modified technique are, therefore, digestion of the sample for a brief period with a relatively high concentration of hydrochloric acid, followed by cooling, oxidation with potassium permanganate, and addition of potassium thiocyanate. Thiocyanate was selected in preference to several other reagents because it yields a satisfactory color reaction with iron in strong acid solution (*3*).

Colorimetric Determination of Iron in Water

The method is designed to measure iron present as soluble salts or suspended oxides in either the ferrous or ferric condition. A small proportion of the iron compounds in soil washings may also be sufficiently soluble in acid to be measured.

If only soluble iron salts are to be determined, the sample should be filtered at the time of collection to avoid precipitation due to loss of carbon dioxide or to oxidation of iron resulting from aeration.

Reagents. All reagents are prepared from chemicals of reagent grade.

Stock Ferric Iron Solution. Dissolve 0.7022 gram of crystallized ferrous ammonium sulfate, $FeSO_4(NH_4)_2SO_4.6H_2O$, in 50 ml. of distilled water and 5 ml. of concentrated sulfuric acid. Add potassium permanganate until the pink color persists for 1 minute. Dilute to 1 liter with distilled water; 1 ml. contains 0.1 mg. of iron.

Standard Ferric Iron Solution. Dilute 10 ml. of the stock solution to 100 ml. with distilled water just before using; 1 ml. contains 0.01 mg. of iron.

Potassium Thiocyanate, Approximately 3 *M*. Dissolve 291 grams of the dry salt in distilled water and dilute to 1 liter.

Hydrochloric Acid, Approximately 4 *M*. Dilute 100 ml. of the concentrated acid, specific gravity 1.18 to 1.20, with 200 ml. of distilled water.

Potassium Permanganate, Approximately 0.2 *N*. Dissolve 6.3 grams in 1 liter of distilled water.

Preparation of Iron Standards. Dilute 0.09-, 0.3- . . . . . 12.0-ml. portions of standard iron solution to 30 ml. with distilled water. Proceed as with samples but omit heating.

Preparation of Permanent Standards. Potassium Chloroplatinate, K_2PtCl_6. Dissolve 4 grams in distilled water. Add 200 ml. of concentrated hydrochloric acid and dilute to 1 liter with distilled water.

Cobaltous Chloride, $CoCl_2.6H_2O$. Dissolve 48 grams in distilled water. Add 200 ml. of concentrated hydrochloric acid and dilute to 1 liter with distilled water.

Cupric Chloride, $CuCl_2.2H_2O$. Dissolve 12 grams in distilled water. Add 100 ml. of concentrated hydrochloric acid and dilute to 1 liter with distilled water.

Procedure. Put the volumes of color solutions specified in Table I into 50-ml. Nessler tubes or 2-ounce (28-mm.) French square bottles, as designated, and make up to 50 ml. with distilled water. Compare these solutions with freshly prepared iron standards and, if necessary, adjust the volumes of color solutions to secure a close visual match with the reagents and lighting conditions used. Repeat this comparison with iron standards whenever new solutions are prepared.

Examination of Samples. Measure 30 ml. of sample into a 2-ounce French square bottle of clear colorless glass, add 20 ml. of 4 *M* hydrochloric acid, and heat in a water bath at a temperature just below boiling for 20 minutes. Allow to cool to room temperature. Add 1 drop of 0.2 *N* potassium permanganate, or enough to maintain a perceptible color for 1 minute. Add 5 ml. of 3 *M* potassium thiocyanate, mix, and compare at once with color standards in identical bottles, using diffuse daylight or light from a fluorescent daylight lamp. If less than 0.4 p. p. m. of iron is present, transfer at once to a 50-ml. Nessler tube and compare with color standards in identical tubes. If the iron concentration is greater than 4.0 p. p. m., repeat the test with a smaller volume of sample diluted to 30 ml. with distilled water.

Solution of Suspended Iron. The proposed method was selected after trial of varying combinations of acidity and heating time, so as to dissolve principally the significant forms of iron. However, no sharp line of demarcation between the two forms of iron based on the ease of dissolution in acid is possible, since soil washings may contain simple oxides, and scale from corroded conduits may be difficultly soluble in acid.

Determination of the Smallest Significant Amount of Iron. A concentration of 0.1 p. p. m. was taken as the smallest significant value; this is the lowest value for which a permanent standard is described in standard methods (*1*), based upon a 100-ml. sample. In the proposed method the determination of iron at this concentration without evaporation of the sample or removal of excess acid was accomplished by increasing the amount of potassium thiocyanate. The effect of such an increase is shown in the curve of Figure 1, which represents the relative proportions of iron and thiocyanate required for equivalent color development. As indicated, 0.003 mg. of iron or 30 ml. of sample at 0.1 p. p. m. and the designated potassium thiocyanate concentration, approximately 0.3 *M*, yields as deep a color as the lowest permanent standard in the standard methods. Another permanent standard of lighter color representing 0.03 p. p. m. with the proposed method has also been found satisfactory for routine use.

Table I. Preparation of Permanent Color Standards

Equivalent Concentration of Iron in 30-Ml. Sample	Platinum Solution	Cobalt Solution	Copper Solution
P. p. m.	*Ml.*	*Ml.*	*Ml.*
For use in 50-ml. Nessler tubes, 240 mm. to mark			
0.03	0.40	0.47	1.00
0.10	0.75	0.80	1.56
0.20	1.25	1.25	1.33
0.30	1.63	1.72	1.15
0.40	1.95	2.17	1.00
0.50	2.20	2.65	0.80
For use in 2-ounce (28-mm.) French square bottles			
0.40	1.90	1.00	0.20
0.60	2.60	1.90	0.80
1.0	4.60	3.80	4.60
1.5	6.90	6.15	6.60
2.0	8.95	8.45	8.00
2.5	11.0	10.8	9.20
3.0	12.6	13.2	10.2
4.0	15.6	17.8	11.6

Table II. Influence of Sodium Hexametaphosphate on Thiocyanate Color Reaction with Iron

(All concentrations expressed as p. p. m. in 50 ml. or molarity)

		Standard Method	Standard Method with Added HCl	Standard Method with Added KSCN	Proposed Method
HCl concentration		0.05 *M*	1.6 *M*	0.05 *M*	1.6 *M*
KSCN concentration		0.02 *M*	0.02 *M*	0.3 *M*	0.3 *M*
Sodium Hexametaphosphate Calculated	Iron Calculated	Iron Observed			
P. p. m.	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*
2	1.0	0.75	0.9	1.0	1.0
5	1.0	0.65[a]	0.9	0.9	1.0
10	1.0	0.4[a]	0.9	0.8[b]	1.0
20	1.0	0.1[c]	0.9	0.6[a]	1.0
2	5.0	4.8	5.0	5.0	5.0
5	5.0	4.3[b]	5.0	4.5	5.0
10	5.0	3.5[a]	4.9	4.2[b]	5.0
20	5.0	2.5[c]	4.8	4.0[c]	4.9

[a] Markedly different hue from that of iron standards; [b] slightly different; [c] so different that accurate reading in terms of standards was impossible.

Influence of Organic Matter. The usual precaution of destroying organic matter by wet or dry combustion is probably essential for an exact determination of iron in many materials. However, in the examination of a few iron-bearing samples of water selected as representing clear waters of high organic content, the proposed method yielded results identical with those of duplicate samples previously evaporated, ignited, and redissolved with the hydrochloric acid reagent. Oxygen-consumed values of these samples were 1.1, 1.9, 4.3, 6.4, and 22.4 p. p. m.; the colors were 5, 7, 90, 180, and 0 p. p. m., respectively. Thus it would appear that the usual forms of organic matter may be present in large amounts and not interfere with the determination of iron in water by this technique. Elimination of this interference appears due to the high acid concentration with which potassium permanganate is used to oxidize ferrous iron. There may be instances when compounds of iron and organic matter are present that do not react with thiocyanate under the conditions of the test. Such iron compounds, however, would, like those in soil washings, be of little interest in problems of water treatment, distribution, or use.

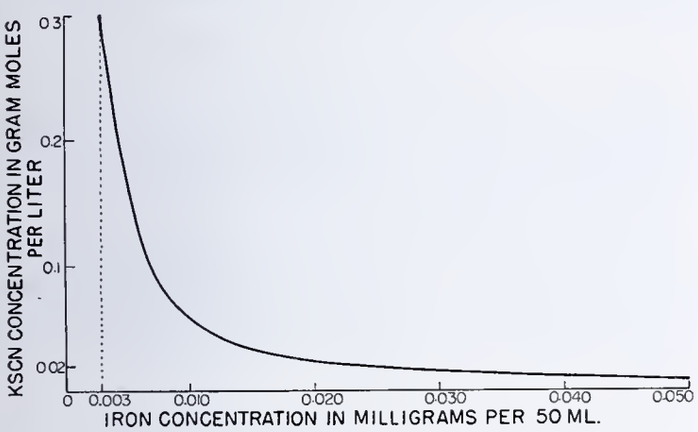

Figure 1. Objective Simulation of Variation of Threshold Sensitivity with Thiocyanate Concentration

Curve represents points giving same transmission through 4 cm. of the solution with light previously passed through No. 54 green filter as measured by a Klett-Summerson colorimeter. All solutions were made to contain 1.6 *M* hydrochloric acid. Transmission selected was that given by 0.01-mg. iron standard prepared as described in standard methods.

Influence of Copper. The thiocyanate reaction with copper is about as sensitive as that with iron. Since the hue of the color developed is markedly different for the two metals, copper interference is evident on comparison with standards if its proportion to iron is high. This difference in hue has often indicated the desirability of examining a particular sample for copper and for iron by a more specific test, such as that suggested by Swank and Mellon (*2*). Whenever the treated samples and color standards are alike in hue, copper may be considered absent or its concentration low compared to that of iron.

Interference by Sodium Hexametaphosphate and by Sodium Pyrophosphate. Various dilutions of sodium hexametaphosphate and of iron were examined for iron with thiocyanate in the concentrations specified in the standard method and in the proposed method, but without heating. Both thiocyanate concentrations were used with the hydrochloric acid concentrations given in each method, making a total of four combinations. All color comparisons were made with iron standards treated with the amounts of reagents used in the hexametaphosphate-treated sample. The results shown in Table II indicate a marked advantage for the proposed method, owing principally to the high acid concentration used.

Table III. Recovery of Iron Added to Natural Waters

Original Water			Original Water Plus 1 P. P. M. of Iron	Recovery of Iron Added
Color[a]	Turbidity[a]	Iron		
P. p. m.	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*
0	0	0	1.00	1.00
27	Trace	< 0.1	1.00	0.95
80	2	0.30	1.20	0.90
10	10	0.80	1.75	0.95
0	25	3.00	4.00	1.00
10	5	1.00	2.06	1.06
28	18	1.30	2.30	1.00
3	5	1.00	2.00	1.00
0	10	1.85	2.88	1.03
0	10	1.27	2.31	1.04
28	7	1.07	2.00	0.93
55	Trace	0.60	1.60	1.00

[a] Determined by procedure in standard methods (*1*).

The colors developed by iron with thiocyanate in the presence of concentrations of sodium hexametaphosphate above 10 p. p. m. varied considerably in a series of replicates, so that the extent of interference at higher concentrations could not be determined accurately. If the solutions of sodium hexametaphosphate and iron are heated as specified in each of the two methods, this interference is markedly diminished, but it is still greater with the standard method. When the proposed method was used with heating, 1000 p. p. m. of sodium hexametaphosphate reduced the color due to 1.0 and 5.0 p. p. m. of iron less than 10 per cent. Similar results were obtained with sodium pyrophosphate with or without heating.

Color Standards. The permanent color standards described in Table I are prepared from solutions of potassium chloroplatinate, cobalt chloride, and copper chloride. The first two materials could be blended satisfactorily for hue alone but all three are essential for both the required hue and brightness. These permanent color standards represent a close visual match for iron standards and are simpler to use. After 6 months of routine use they were also a close visual match for freshly prepared standards.

Precision. The precision of the method is illustrated by the recovery experiments in Table III. In each case the value found for iron compared favorably with the amount added.

Discussion

The proposed method has a number of advantages over the standard method (*1*), particularly when large numbers of samples are examined routinely. It obviates the segregation

of silt-bearing waters and of those of varying organic content for separate treatment. Since the hydrochloric acid used to dissolve suspended iron is not removed by evaporation, no fume hood is required. Hydrochloric acid is used rather than nitric acid, since the latter decomposes on storage unless protected from light. The deep color obtained by adding a large amount of thiocyanate permits the use of a comparatively small sample. When relatively large amounts of thiocyanate and acid are used, the hue and intensity of color produced with the iron are not affected by moderate variations in concentration. The 2-ounce French square bottles used for the test are inexpensive, may be stored compactly, and are subject to relatively slight loss from breakage. The attention required when individual samples are boiled with acid or evaporated and redissolved in acid is avoided by heating in a water bath under conditions that involve no change in volume or acidity. Interference by copper is approximately the same for both methods, but interference due to sodium hexametaphosphate or sodium pyrophosphate is negligible. Compared with a number of other color reactions for iron, the one described is simpler, since it is applicable in strong acid solution.

Summary

The simple colorimetric thiocyanate method described for the determination of iron in water is applicable in the presence of amounts of organic matter, sodium hexametaphosphate, or sodium pyrophosphate greater than are likely to be encountered. The test is not applicable in the presence of copper; in such instances it constitutes a qualitative test for this material. The color standards are stable for at least 6 months.

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Concentration of Hafnium

Preparation of Hafnium-Free Zirconia

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THE development of the chemistry of hafnium since its discovery in 1923 has not been particularly extensive. The lack of a convenient method for the separation of hafnium from zirconium has undoubtedly been the principal deterring factor. Previous methods have involved fractional crystallization of the hexafluorides (*2*) or oxychlorides (*4*) and fractional decomposition of complex ions of zirconium-hafnium formed with sulfuric acid, phosphoric acid, hydrofluoric acid, dicarboxylic acids, alpha-hydroxycarboxylic acids, and polyhydroxy alcohols (*1*) to yield the oxides, phosphates, or ferrocyanides (*10*) as precipitates.

These methods require a large number of fractional separations, entail the handling of highly corrosive solutions, or utilize expensive reagents, all of which make large-scale operations impractical. The direct precipitation of the phosphate in dilute acid solution has never been carried through for the fractionation of zirconium-hafnium compounds, although it is known that hafnium phosphate is less soluble than zirconium phosphate (*3*, *5*, *8*).

The utilization of the phosphates is handicapped by the gelatinous character of the usual phosphate precipitate, which results in slow filtration and inefficient washing, and by the lack of a convenient method for converting the insoluble phosphate into soluble compounds for reprecipitation. Both these difficulties have been overcome and a more satisfactory method for the concentration of hafnium oxide has been developed.

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Source of Material

The ore used in this investigation was cyrtolite, an altered zircon which conforms approximately to the formula $R_3Y_2(Zr,Hf)(SiO_4)_{12}$. It was obtained from deposits near Bedford, N. Y., and Hybla, Ontario.

Ordinarily silicates are not easily attacked by acid treatment, and must be handled by some fusion method. Urbain (*9*), however, reported successful extraction by sulfuric acid at 65° C. of malacon, an altered zircon. Recently Schumb and Pittman (*10*) noted that cyrtolite yielded to sulfuric acid treatment, but gave no details of the procedure. Van Osdall (*11*) made preliminary investigations on the acid "cracking" of cyrtolite, which resulted in satisfactory yields.

To be susceptible to acid attack, the ore must be in a finely divided state. When material of particle size larger than that separated by 100- or 200-mesh screens is treated with concentrated sulfuric acid, the yields are not satisfactory. After an exhaustive series of quantitative experiments to determine the optimum conditions for the acid cracking of the ore, the following procedure was found to give nearly 100 per cent extraction of the zirconium-hafnium content in a minimum of time.

One hundred grams of 200-mesh ore and 200 grams of concentrated sulfuric acid are mixed in an evaporating dish and placed on a hot sand bath. After about 20 minutes the temperature reaches a maximum of 210° to 220° C. and the mixture begins to thicken. The heating process continues for about 10 minutes, or until a stiff mud results. At this point heating is discontinued, and the "cracked" ore is cooled and added with stirring to 500 ml. of water. The addition of 10 ml. of 10 per cent glue solution aids in coagulation and filtration of the insoluble material. The solution is filtered and the residue on the funnel washed with water. The filtrates are combined. Treatment of the residue with sulfuric acid under the same conditions does not result in any further loss of weight.

Table I. Determination of Hafnium Oxide

HfO_2 by Density	HfO_2 by Selenites
%	%
16.8	17.1
23.2	23.6
50.4	51.0
87.8	93.3
89.0	97.7

Method of Analysis

Because of the large number of zirconium-hafnium samples to be analyzed, a rapid method of analysis was desired. Of the methods available at the time this work was started, the density determination of the ignited oxides seemed most satisfactory. Difficulties with adsorbed gases and the preparation of the samples were overcome by the following procedure:

Sufficiently purified zirconium-hafnium solution is taken to provide 1 to 1.5 grams of the ignited oxide. (The sample is purified by the solution of the hydrated oxides in concentrated hydrochloric acid and subsequent crystallization of the oxychlorides. The iron is removed by washing the oxychlorides with ether.) The hydrated oxide is precipitated from solution with ammonium hydroxide, washed free of sulfate ion, and ignited over a Fisher burner (ca. 900° C.) to constant weight. The oxide is ignited once more, transferred to a weighed pycnometer, and the weight of the sample determined. The oxide is covered with about 1 ml. of freshly boiled distilled water and the pycnometer is then placed in a vacuum desiccator where it remains until gas bubbles no longer rise from the surface of the oxide. It is assumed that the oxide has been degassed when bubbles cease to be eliminated. The pycnometer is then filled with water and the thermometer inserted. The apparatus is placed in a constant-temperature bath at 25.00 ± 0.01° C. and allowed to come to equilibrium. The liquid level in the side arm is brought to the calibration mark, and the pycnometer is removed from the bath and weighed when dry.

Table II. Precipitation of Zirconium Phosphates

RO_2 Precipitated Theory	RO_2 Precipitated Actual	Ratio of Actual to Theory
%	%	
	Van Osdall Method	
10.0	14.3	1.43
15.0	22.2	1.47
22.5	38.4	1.55
35.0	56.6	1.64
	Proposed Method	
50.0	51.7	1.03
58.0	62.4	1.08
66.0	69.2	1.05

When this procedure is carefully followed, the densities of different samples prepared from the same solution are reproducible to ±0.02. The per cent of hafnium oxide is calculated from the following formula (*6*):

$$\text{Per cent } HfO_2 = \frac{d_{\text{mixt.}} - d_{ZrO_2}}{d_{HfO_2} - d_{ZrO_2}} \times \frac{d_{HfO_2}}{d_{\text{mixt.}}} \times 100$$

The values for the densities of pure zirconium oxide and pure hafnium oxide prepared by ignition of the normal sulfates have been reported by von Hevesy (*6*) as 5.73 and 9.74, respectively. A sample of hafnium-free oxide prepared by the ignition of the hydrated oxide over a Fisher burner gave a value of 5.62 ± 0.02 rather than 5.73 for the density. When ignited in a muffle furnace for 2 hours at 900° C. a density of 5.64 was obtained. Since a low value for the density of zirconium oxide was obtained, it was assumed that a low value for the density of hafnium oxide would also result from this method of preparation. Thus the value of 9.67 as previously reported by von Hevesy (*7*) was selected and used in all calculations, with 5.62 for zirconium oxide.

In order to have an independent check upon the per cent of hafnium oxide present in any sample, the method of analysis described by Schumb and Pittman (*10*) was used. This involves precipitation of the basic zirconium-hafnium selenites, digestion to the normal selenites, $(Zr, Hf)(SeO_3)_2$, and ignition to the oxides of a washed and dried sample of the mixed selenites. In all cases the values obtained by the selenite method (Table I) are higher, but fall within the accuracy of the methods except in the hafnium oxide–rich region. (The relation of time and temperature of ignition to density, crystal structure, and composition of various zirconium oxide–hafnium oxide mixtures is now under investigation.)

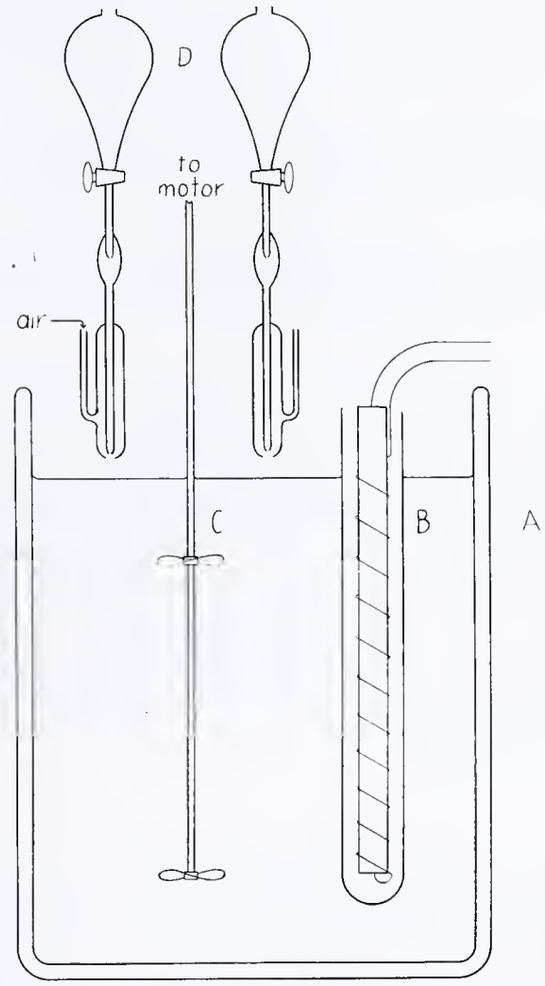

Figure 1. Apparatus Assembled for Precipitation

Precipitation of Zirconium-Hafnium Phosphates. When phosphoric acid is added to a zirconium salt solution, a voluminous gelatinous precipitate results. It adsorbs (or occludes) large quantities of mother liquor and is almost impossible to filter. An improved method of precipitation was developed by Van Osdall (*11*) in which 12 to 15 per cent phosphoric acid was added dropwise to a hot (85° to 90° C.), dilute (3 per cent zirconium oxide) solution of zirconyl sulfate. When this method of precipitation is used, a granular precipitate results which filters readily, but an appreciable quantity of mother liquor is still adsorbed, as indicated in Table II.

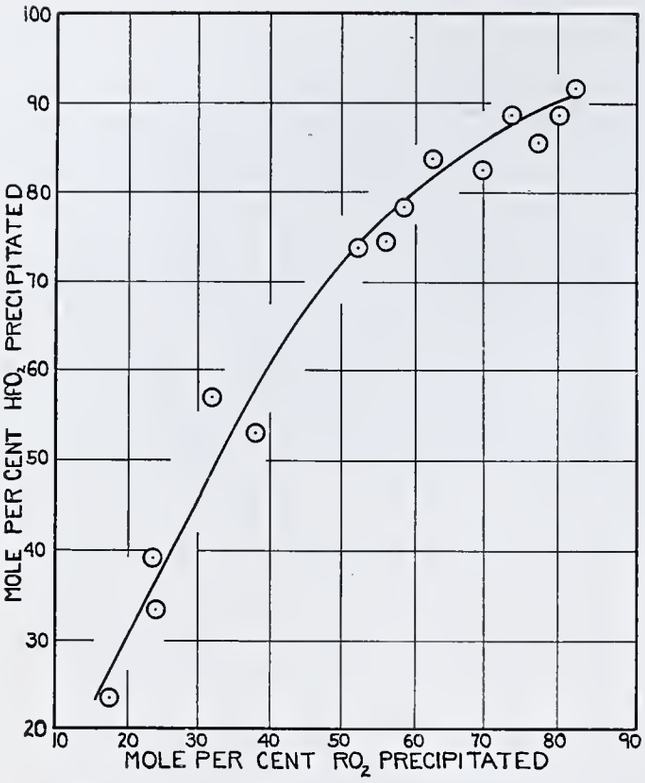

Figure 2. Relationship between Hafnium Oxide Content of Precipitate and Fraction of Total Hydrated Oxides Precipitated as Phosphates

In other words, for every gram of oxide precipitated as the phosphate, about one half of the oxide (as the sulfate) is adsorbed. The adsorbed material is difficult to remove by washing. Thus the efficiency of the separation is reduced, since the adsorbed material is not fractionated. When a drop of phosphoric acid strikes the surface, the precipitate forms as a globule which may well enclose mother liquor in the center. These particles can be broken up by vigorous stirring, but then a precipitate which is difficult to filter is obtained. To eliminate these difficulties the following method of precipitation was developed which reduces adsorption to a minimum (Table II) and gives a dense, compact, easily-filtered precipitate:

Three solutions are prepared, (1) 10 per cent sulfuric acid, (2) a 2 to 5 per cent phosphoric acid solution in 10 per cent sulfuric acid, and (3) a zirconyl sulfate solution containing 2 to 5 per cent zirconium oxide also in 10 per cent sulfuric acid. Instead of addition of phosphoric acid to a solution of zirconyl sulfate, the reagents are added simultaneously, by means of an atomizer arrangement, to 45 liters of 10 per cent sulfuric acid, in a suitable earthenware crock (*A*, Figure 1). The acid liquor is heated to 75° C. by a Vitreosil immersion heater, *B*. The solution is mixed with a motor-driven stirrer, the shaft and paddles, *C*, of which are constructed of acid-resistant Hastelloy C. Care must be taken to add both reagents at an equivalent rate. The rate of addition for the zirconyl sulfate solutions which seems best is 1000 to 1200 ml. per hour. The rate of flow can best be determined by means of a drop counter inserted below the stopcock of a separatory funnel, *D* (Walther funnel). At 75° C. the rate of evaporation almost equals the rate of addition of the reagents; therefore, a constant volume of solution is maintained. The net result of the reaction is to increase the acid concentration from 10 to 13 per cent sulfuric acid.

The best precipitate is obtained when the final suspension contains 35 to 200 grams of phosphates per 10 liters of mixture. At lower concentrations the precipitate again becomes flocculent rather than granular and the time of filtration and the amount of mother liquor adsorbed increase. At higher concentrations, the suspension becomes so heavy as to slow down filtration. If the above conditions are observed, the precipitate settles so rapidly that the major portion of the acid liquor can be removed by decantation within a short time. One complete phosphate precipitation accompanied by thorough washing of the precipitate will eliminate most of the uranium, iron, manganese, and rare earth impurities.

Conversion of Phosphates to Acid-Soluble Compounds. In order to proceed with the fractionations, the zirconium-hafnium phosphates must be converted to acid-soluble compounds. It is the elimination of this difficulty which has led to methods of separation which involve complex ions. It was found that treatment of the moist phosphate with solid sodium hydroxide results in about 80 per cent conversion of the phosphate to the hydrated oxide. In an attempt to produce more complete conversion, sodium peroxide was added to the phosphate suspension in the presence of an excess of base. Partial solution of the phosphate resulted, probably through the formation of soluble peroxyzirconates and hafnates. Upon digestion of this solution a dense white precipitate was obtained. This is apparently a peroxide, since a washed sample liberates iodine from potassium iodide in acid solution. Reaction of the phosphates with sodium peroxide alone resulted in only 76 per cent conversion. To establish the best conditions, various sodium hydroxide–sodium peroxide ratios were added to a definite quantity of zirconium phosphate and the per cent conversion was determined.

The following procedure for the conversion of the phosphate to the peroxide hydrates was finally decided upon:

The filtered phosphate is mixed thoroughly with water to give a slurry which contains about 150 grams of phosphate (dry basis) per liter of water. Any adsorbed acid is neutralized (litmus) with sodium hydroxide solution. The slurry is then cooled with ice to 0–10° C. The sodium hydroxide (2 moles per mole of ZrP_2O_7) is dissolved in water to give a 50 per cent solution, and cooled to 0° C. with ice. To this cold solution is added the sodium peroxide (2.3 moles per mole of ZrP_2O_7); at this temperature little decomposition of the sodium peroxide occurs. The alkaline peroxide solution is added with stirring to the phosphate slurry. At this point the resulting mixture will contain 60 to 100 grams of the phosphate per liter of solution. It is then digested on the hot plate at 50° to 70° C. for about 3 hours until the intermediate soluble peroxyzirconate is completely decomposed to the insoluble peroxide hydrate. Heating to higher temperatures will reduce the time of conversion, but the character of the precipitate makes filtration more difficult. The solution containing the peroxide must be filtered while hot to avoid crystallization of disodium hydrogen phosphate. The phosphate-free peroxide hydrate is dissolved in sulfuric acid solution. A clear solution results if the precipitate has been washed free of phosphate. The washing is rapid and complete when the solution has been properly digested.

Fractionation of Zirconium and Hafnium. The mechanics of the method of fractionation is the same as that for complete precipitation, except that an insufficient amount of precipitant is used. The number of grams of phosphoric acid necessary to precipitate the desired per cent of RO_2 as the phosphate is diluted with 10 per cent sulfuric acid to give the same volume as that of the zirconyl sulfate. The two solutions are then added to a 10 per cent sulfuric acid solution at the same rate. At times it is more convenient to dilute the phosphoric acid to only a fraction of the volume of the zirconyl sulfate solution to prevent excessive dilution of the acid; the rate of addition of the phosphoric acid must then be correspondingly reduced. Care must be taken that the rate of addition of the reagents is such that a constant fraction of the total RO_2 is removed throughout the precipitation.

A series of fractionations was carried out in which the fraction of the total oxides precipitated as the phosphates in the first precipitation was varied from 20 to 80 per cent. The corresponding mole fraction of hafnium removed in the first

precipitation was then determined. These data, plotted in Figure 2, show that the most efficient point for separation is in the region where 35 to 45 per cent of the total oxides are precipitated as the phosphates. It is in this region that the concentration factor—i. e., the ratio of hafnium oxide in the precipitate to the hafnium oxide content in the original—is at a maximum. The concentration factor seems to be independent of the original hafnium oxide content over the range 10 to 75 per cent hafnium oxide. However, if it remained constant in the higher hafnium oxide range, then within a few fractions all of the hafnium should appear in the precipitate. This result is contrary to experimental observations of similar fractionations of rare earths and radium. Unfortunately, sufficient reliable data are not available to determine the change in the concentration factor in the region above 75 per cent hafnium oxide.

If higher percentages of the total oxides are precipitated, the yield of hafnium oxide increases but the number of fractionations necessary also increases. If small percentages are removed, the yield of hafnium-rich fractions decreases rapidly.

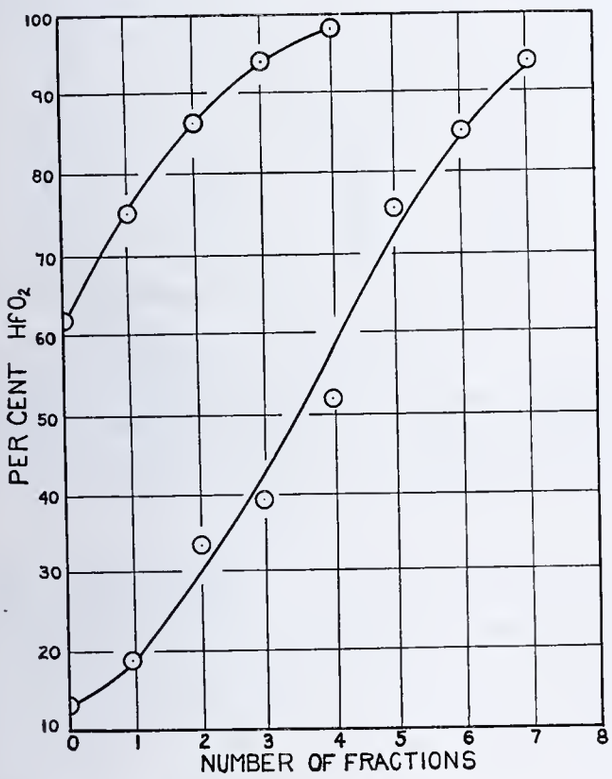

Figure 3. Increase in Hafnium Oxide Concentration with Successive Fractionations

Simultaneously with the above experiments, a separation series was run in which the fractions precipitated averaged 55 per cent of the total RO_2 of the preceding fraction. The progress of the separation can be followed in Figure 3. In seven successive fractions, in which the mother liquors were set aside, the hafnium content of an oxide mixture was increased from 13 to 93 per cent. The yield of hafnium-rich oxide was about 10 per cent of the hafnium in the original sample. In four successive fractionations of a sample of hafnium oxide concentrates, the hafnium-oxide content was increased from 59 to 97.7 per cent with a yield of about 30 per cent. Mother liquors of approximately equivalent concentration from the various steps were combined for retreatment, using the procedure outlined. The purpose of the retreatment was to obtain further zirconium-rich and hafnium-rich oxides.

To obtain hafnium-free zirconia, a sample of low hafnium content (2 to 3 per cent) was fractionated by two successive steps in which about 60 per cent of the total oxide was precipitated as the phosphate. The remaining mother liquor, when purified by oxychloride recrystallization, gave a zirconium oxide free of hafnium as shown by arc spectrum analysis.

Summary

A satisfactory extraction of the cyrtolite ore is obtained when two parts of concentrated sulfuric acid and one part of 200-mesh ore are heated to a maximum temperature of 210° to 220° C., and the resulting acid-soluble constituents are extracted with water.

When acid solutions of dilute zirconyl-hafnyl sulfate and dilute phosphoric acid are simultaneously sprayed into a 10 per cent sulfuric acid solution at 70° to 75° C., a dense, compact, and easily filterable precipitate is obtained.

By the action of an ice-cold sodium hydroxide–sodium peroxide solution upon a cold slurry of the phosphates, and subsequent digestion at 70° C., an acid-soluble hydrate results. This step replaces the usual basic fusion or complex-ion formation as the intermediate step between fractionations.

The conditions for the fractional separation of zirconium and hafnium by direct precipitation of the phosphates in dilute solutions were studied. By precipitating approximately 55 per cent of the dissolved zirconium-hafnium oxide content of a sulfate solution, the percentage of hafnium oxide of a sample of the mixed oxides is raised from 13 to 93 per cent in seven fractionations, and from 59 to 97 per cent in four fractionations. By treating the mother liquors from the precipitations in similar fashion, a sample of zirconium oxide whose spectrogram revealed no lines characteristic of hafnium is obtained.

Acknowledgment

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Determination of Sodium in the Presence of Molybdenum

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ALTHOUGH several publications (*2, 3, 5, 7*) have dealt with the interferences encountered in the determination of sodium by the zinc uranyl acetate method of Barber and Kolthoff (*2, 5*), no reference is found to the effects of molybdenum. Molybdate interferes by the formation of a precipitate with the zinc uranyl acetate reagent. This precipitate, which is a yellow, amorphous material, is apparently the uranyl molybdate, UO_2MoO_4, described by Lancien (*6*).

The interference of the molybdate may be either positive or negative. If the precipitate is retained on the filter and weighed with the sodium, the results will be high. If the precipitate, which is usually very fine, passes completely through the filter, the correct value may be obtained unless the depletion of reagent by its reaction with the molybdate causes incomplete precipitation of the sodium, in which case the values will be low. These interferences are illustrated in Table I.

The removal of the molybdenum by means of hydrogen sulfide is tedious and time-consuming, especially since repeated precipitations are often necessary (*8*). The use of complex ions to effect the removal of interferences is common in analytical chemistry. Of the complexes that may be formed with molybdenum, phosphate precipitates the reagent; fluoride retards the formation of the sodium zinc uranyl acetate; and the oxalate complex is not sufficiently stable. The tartrate and citrate complexes, however, are both stable (*1, 4*) and do not interfere. Although, as Barber and Kolthoff stated (*2, 5*), an excess of such organic acids precipitates the reagent, the concentration necessary to effect the removal of the molybdate is not large enough to cause interference.

Table I. Effects of Molybdenum on the Determination of Sodium

Na_2O Added	MoO_3	Na_2O Found
Gram	*Gram*	*Gram*
0.0020	0.0	0.0020
0.0020	0.01	0.0023
0.0000	0.1	0.0046
0.0020	0.1	0.0021
0.0020	0.1	0.0002
0.0020	0.1	0.0043

Experimental

The method used is a modification of that introduced by Barber and Kolthoff (*2*). The solution, slightly acid with sulfuric acid, is oxidized with a drop of nitric acid and the molybdate is converted to the complex by the addition of citric or tartaric acid. The sodium is then precipitated by the addition of an excess of zinc uranyl acetate reagent.

Reagents. Tartaric or citric acid solution. Dissolve 50 grams of the acid in 50 ml. of water.

Zinc uranyl acetate solution. Dissolve separately (*A*) 308 grams of uranyl acetate dihydrate in 1640 ml. of water and 54 ml. of glacial acetic acid, and (*B*) 924 grams of zinc acetate trihydrate in 1048 ml. of water and 27 ml. of glacial acetic acid. Mix solutions *A* and *B* at 70° C., and allow to stand for 24 hours at 20° C. Filter immediately before use.

Procedure. The sample, in solution as sulfates with a slight excess of sulfuric acid, is concentrated to as small a volume as possible without crystallization (10 ml. or less). Add one drop of concentrated nitric acid to oxidize any molybdenum that has been reduced during the concentration, and 0.2 ml. of 50 per cent tartaric or citric acid solution for each 0.1 gram of molybdic oxide present. Filter into the concentrated solution a tenfold excess of zinc uranyl acetate reagent, stirring the solution vigorously during the addition of the reagent to prevent crystallization. Allow the sample to stand, with occasional stirring, for one hour at 20° C., and filter through a tared filtering crucible. Wash 5 to 10 times with 95 per cent ethyl alcohol saturated with sodium zinc uranyl acetate, and finally twice with ether or acetone. Dry in a desiccator for 10 minutes and weigh. Correct for a blank determination carried out in the same manner.

Weight of Na_2O = weight of precipitate × 0.0202.

Data. Table II summarizes the results of the analyses of a number of solutions prepared by adding measured volumes of a standard sodium sulfate solution to varying amounts of molybdenum.

Table II. Determination of Sodium in the Presence of Molybdenum

Na_2O Added	MoO_3	Na_2O Found	Error
Mg.	*Mg.*	*Mg.*	*Mg.*
0.4	500	0.4	0.0
2.0	100	2.1	+0.1
2.0	200	2.1	+0.1
2.5	100	2.5	0.0
4.0	100	4.0	0.0
8.0	100	8.2	+0.2
10.0	100	10.1	+0.1
10.0	500	9.9	−0.1
50.0	100	50.0	0.0

To prove the applicability of the modification to the determination of sodium in aluminous materials containing molybdenum, known amounts of the sodium sulfate solution and molybdenum oxide were added to excesses of aluminum hydroxide. The results of the analyses of these samples are shown in Table III.

Discussion

The results that were obtained showed the same degree of accuracy and precision as analyses of similar materials that contained no molybdenum. The size of sample used for an analysis should be such that the amount of molybdenum does not exceed 0.5 gram of molybdic oxide because of the difficulty of concentrating the solution to 10 ml. or less without the precipitation of molybdic acid. In the presence of potassium salts, citric acid is preferable to tartaric because of the limited solubility of potassium tartrate.

Summary

Molybdenum interferes with the determination of sodium by the zinc uranyl acetate method by the formation of a precipitate with the reagent. This interference can be

Table III. Determination of Sodium in the Presence of Molybdenum and Aluminum

Na_2O Added	MoO_3	Al_2O_3	Na_2O Found	Error
Mg.	*Mg.*	*Mg.*	*Mg.*	*Mg.*
0.5	100	1000	0.6	+0.1
0.6	100	1000	0.6	0.0
0.7	100	1000	0.7	0.0
1.2	100	1000	1.4	+0.2
1.3	100	1000	1.3	0.0
1.6	100	1000	1.6	0.0
2.1	100	1000	2.0	−0.1
2.6	100	1000	2.5	−0.1

overcome by the formation of a complex of molybdenum with citric or tartaric acid.

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Analysis of Commercial Oil Emulsions and Wax Dispersions

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Oil emulsions, which may or may not contain suspended solids, are analyzed by first distilling off any water-immiscible solvent. Benzene is then added and distilled with continuous return, carrying with it the water which does not return to the sample. This avoids formation of troublesome emulsions during the conventional extractions of the oil phase. This procedure is sufficiently longer than the conventional extraction to be inadvisable for simple oil emulsions if they are readily extractable with ether. The aqueous distillate contains any alcohol. By centrifuging if necessary and evaporation of benzene, the oil is recovered without undue oxidation. The solid residue is then separated by conventional methods into alcohol-soluble, chlorohydrocarbon-soluble, water-soluble, and mineral fractions for separate analysis. Amine emulsifiers are determined on another portion of the original sample but all other determinations are carried out on the single sample.

Improved methods for analysis of water-base wax dispersions provide for precipitation by acid, followed by separation and approximate identification of resins, wax, amines, etc.

METHODS of analysis of emulsions and suspensions as previously published (*2*) have been revised with further experience to simplify the procedures and provide greater accuracy. One major change has been in methods applied to oil emulsions, which may or may not contain suspended solids, to give more complete separation, avoid troublesome extractions in a separatory funnel, and prevent oxidation of the oils present. The importance of water-base wax suspensions has increased steadily and the types of soap stabilizers used have been complicated by commercial introduction of numerous amines. For brevity many conventional methods of analysis are referred to only briefly without details.

Oil Emulsions

The products in this class may vary from a medicinal oil emulsion to automobile cleaner-polishes. Many of the products contain fractions, such as petroleum naphtha, which are volatile with steam. Glycerol is often present, alcohol occasionally. Some products contain from a trace to 2 per cent of waxes. The emulsifying agents encountered individually or as mixtures include 1 to 5 per cent of bentonite, varying with consistency and type of clay, usually less than 1 per cent of various gums, and surface-active agents, usually sulfated oils or soaps. The latter are apt to be amine salts of fatty acids, but occasionally the other members of the class are present.

Persistence of soap and gum emulsions is a problem in conventional extractions which to a greater or lesser degree extends to other surface-active agents. Of all the emulsifying agents mentioned, bentonite is the worst offender. Ether-water emulsions stabilized with it will often not break even on prolonged centrifuging or on addition of minor amounts of alcohol (*1*).

The method provides for first distilling any solvent volatile with steam, then adding benzene and distilling water with it. The oil, emulsifying agent, and abrasive, if any, remain in the flask in excess benzene and receive more conventional treatment. Only a single sample is required unless a nitrogen determination is required for estimation of amines.

Outline of Separation of Commercial Oil Emulsions

Original sample	
Steam distillation	— Immiscible solvent, water, alcohol
Reflux with benzene	— Water, sometimes alcohol
Centrifuge cold	— Oils and sulfated oils in benzene solution
Extract with hot alcohol	— Glycerol, soap, fatty acid in alcohol
Extract with hot $C_2H_3Cl_3$	— Waxes in $C_2H_3Cl_3$
Extract with hot water	— Water-soluble gums
Mineral matter	

Generally speaking, it is not necessary to use this method if the emulsion is of oil in water without added mineral mat-

ter and the emulsion will break down readily with ether or petroleum ether.

Volatile Water-Immiscible Solvent. Weigh out 100 grams of the sample into a 500-ml. round-bottomed flask to an accuracy of 0.1 gram. Assemblies such as are shown in Figure 1, but using all ground-glass joints, are preferable. Connect the flask to the receiver, Figure 1, *A*, and in turn connect the receiver to a water-cooled condenser. Immerse the flask in a cold oil bath, avoiding possible future overheating by making sure that it does not touch the bottom of the path. If the water content of the sample is low, add a measured volume, such as 50 ml. of water before connecting up the apparatus. Gradually heat the bath and distill over any volatile solvent. Continue to distill, with the water returning to the flask, until no more volatile solvent comes over. Collect the volatile solvent, usually petroleum naphtha, in the graduated tube and read its volume. Allow the apparatus to cool, draw off the water from the bottom of the receiver, and return it to the flask. Collect the volatile solvent fraction, and identify by general tests such as specific gravity and boiling point supplemented by odor or other specific reactions. If volatile solvents with gravities greater than water are present, such as the chlorohydrocarbons, use the receiver shown in Figure 1, *B*. In that case read the volume of the solvent, withdraw it through the stopcock, and identify as usual. As in the previous case return the water in the receiver to the flask.

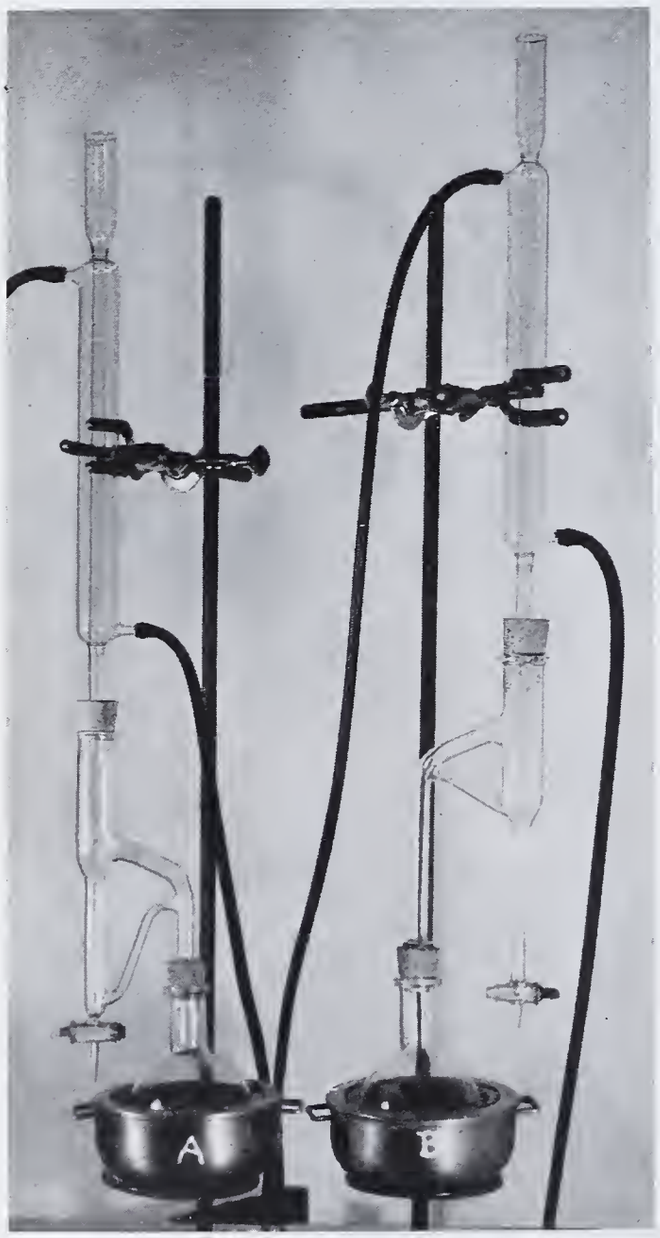

Figure 1. Distillation Apparatus for Use in Analysis of Emulsions

If the sample is known to be free from volatile solvent, these steps can be omitted and the procedure of the following paragraphs applied directly to the sample as originally weighed out.

Water. Add 100 ml. of benzene to the flask and connect to the receiver shown in Figure 1, *B*. Using the same oil bath, reflux the benzene as rapidly as convenient but avoid overheating. Water distills over with the benzene and collects in the bottom of the receiver, while the benzene returns to the flask. At intervals, draw off the water quantitatively into a 50-ml. or 100-ml. volumetric flask. The process of distilling off all of the water from the flask takes about 2 to 3 hours in the average case, but that is largely offset by the fact that the operation is automatic once the size of the burner flame is adjusted. A constant level of the water in the graduated receiver indicates that all the water has distilled over. Weigh or measure the volume of water so collected, subject to correction for alcohol, if present. Deduct any water added to distill water-immiscible solvent.

Alcohols. Dilute the combined aqueous distillates to 50 or 100 ml. and obtain the specific gravity at a known temperature. If alcohol is present, it will be in this distillate and the amount can be calculated from specific gravity tables. Rarely is this other than methanol, ethanol, or isopropanol, which are readily identified by odor, Schiffs' reagent, or the iodoform test. The method is particularly applicable to determination of small volumes of alcohol.

Separation of Oils and Benzene-Insoluble Solids. The oils are now in benzene solution. Thoroughly agitate the contents of the flask to suspend the sediment and transfer to a 250-ml. centrifuge bottle. Rinse out the flask into the bottle with a few milliliters of benzene, and centrifuge the mixture. No water being present, a sharp separation occurs. Decant off the benzene solution and add more warm benzene. Thoroughly break up the solids caked in the bottom of the bottle, shake vigorously to wash this with the benzene, and centrifuge. Repeat the process until the benzene extract is colorless. Usually a total of three or four extractions is sufficient.

Oils. Combine the benzene extracts and evaporate carefully, a convenient technique being to collect the benzene in an empty Soxhlet tube. Evaporate the final residues of benzene on a water bath and dry the oils for a few minutes in an oven, avoiding overheating with consequent oxidation or loss by evaporation. Cool and weigh the oils.

Allow the oil fraction to stand overnight in a desiccator. If saponifiable oils, castor or sulfated castor, for example, are present they will settle out as a viscous layer below the mineral oil. Chill the flask and contents, add chilled petroleum ether on the surface of the extract, and swirl carefully to dissolve the mineral oil. Decant and repeat the operation as necessary. Cold, viscous, saponifiable oil will not appreciably dissolve in chilled petroleum ether. Evaporate off the petroleum ether from these flasks to obtain the mineral and saponifiable oils separately. Analyze these in the usual manner.

Alcohol-Soluble Matter. Break up the residue in the centrifuge bottle and shake well with 75 ml. of warm 95 per cent alcohol (S. D. 3A will do). Cool to room temperature and centrifuge. Decant the alcohol and repeat the process twice more. Evaporate the combined alcohol extracts, finally heat in an oven, and weigh. The treatment of this residue depends largely on the indications of physical examination. A light-colored viscous liquid will usually prove to be glycerol, identified by specific gravity and the acrolein test. In that case there are usually indications during drying, and heating to fumes must be avoided.

In case an alkali-metal soap was originally present, which is rather unusual for this type of emulsion, take up the residue in hot water and titrate with standard hydrochloric acid to a methyl orange end point. Extract the fatty acids derived from the soap with ether and identify in the usual manner. Unless the soap present checks by calculation with the entire amount of alcohol-soluble matter, evaporate the aqueous solution remaining after extraction of fatty acids to dryness. This will contain alkali-metal chloride and any alcohol-soluble material other than soap. Extract this with absolute alcohol to obtain the nonsoap portion of this fraction. Evaporate the alcohol and proceed with tests on this. It will usually be glycerol.

If an amine soap was originally present, it will usually have been decomposed in the benzene distillation and subsequent extraction and drying. In that case an equivalent amount of fatty acid will be present. Take up the residue in warm alcohol and titrate to a phenolphthalein end point. Then evaporate to dryness, take up with warm water, acidify, and extract the fatty acids with ether for identification. If this does not check with the total residue, a separation of glycerol as described in the preceding paragraph may be necessary.

Waxes. To the residue after the alcohol extraction add hot trichloroethylene, break up the abrasive, and shake thoroughly.

Centrifuge while still hot. Decant and repeat the process twice more. Evaporate the combined trichloroethylene extracts. Finally heat to constant weight at 110°. Identify the extracted waxes by odor on burning, melting point, and other constants, if necessary. If the product is a polishing emulsion, it is probable that the wax will be carnauba.

Water-Soluble Gums. After extracting the waxes, drive off any excess trichloroethylene from the centrifuge bottle, first on a water bath and then in the oven. Add boiling water to the hot centrifuge bottle, break up the residue, and shake thoroughly. Centrifuge while hot and decant the solution of gums. Repeat the process at least twice more, always using water near the boiling point. Combine and evaporate the water extracts and obtain the water-soluble gums. It is often convenient to dilute the extracts to a known volume and obtain total solids on an aliquot, testing for the type of gum present on a part or all of the balance. The most probable gums in order of decreasing importance are tragacanth, karaya, and acacia, but there are numerous other possibilities.

Mineral Matter. The residue in the centrifuge bottle is now matter insoluble in benzene, alcohol, trichloroethylene, and water. It will usually be mineral matter, commonly an abrasive, bentonite, or both. Dry the bottle in the oven, conveniently overnight, cool, brush out, and weigh. Observe microscopically for the type of mineral present, or analyze chemically. A mesh analysis is often useful to classify the quality of an abrasive. The absence of other emulsifying agents at earlier steps suggests the probable presence of bentonite at this point.

Amines. Determine nitrogen by the Kjeldahl method on an original sample and in the absence of other nitrogen compounds calculate to the amine suspected. For further details see wax dispersions below.

Water-Base Wax Dispersions

The so-called no-rubbing floor waxes are the most widely distributed examples of this type of product. Rubber finishes, some shoe polishes, and several other types of products are of similar composition. Owing to the complexity of these products and particularly to the extreme difficulty in separating added soap, soaps formed from resins present, resins of many types, and carnauba and other waxes, only approximate figures can be expected from an analysis of this type. Much knowledge of what to expect is needed, to secure reasonably accurate information.

Total Solids. Rapidly weigh 2 to 3 grams of the sample into a weighed porcelain capsule crucible approximately 5 cm. in diameter and 1 cm. deep. Heat the capsule and sample on a steam bath for about 1 hour, and then in an oven at 105° to 110° for 3 hours. Cool and weigh. As the amines normally used in these products vary in rate of volatility, this size of sample, size of dish, and heating time are specified as a compromise found in practice to give best results.

Ash. Ash the nonvolatile matter in the same capsule over a low flame, taking care to ignite off all the carbonaceous matter. Cool and weigh. It is usually then desirable to ash the solids from about 20 grams of sample to provide a larger amount of ash. Dissolve the ash in water, dilute to 100 ml., and test qualitatively. If phosphate or carbonate is present, titrate an aliquot to phenolphthalein and methyl orange end points. Determine any borax on an aliquot by titrating with standard alkali to a phenolphthalein end point in the presence of mannitol or neutral glycerol, applying a suitable correction for other alkaline salts. If other alkaline salts are present phosphate is best determined on another aliquot, using the volumetric molybdate method. Carbonate can be determined by the evolution method using another ashed sample, but is more commonly estimated from the total titration figure after subtracting the titrations equivalent to any borax or phosphate. Any substantial amount of sodium carbonate is usually derived from a soap used as stabilizer. Potassium soaps are rarely used for this purpose.

Acid-Insoluble Matter. Titrate a 100-gram sample, hot, to a methyl orange end point. This will give the total alkali in the sample. Add about 1 ml. of 0.5 N acid in excess and heat to clear the solution thoroughly. Allow to cool, when the waxes, resins, and fatty acids will separate as a waxy mass. Decant off the water layer and wash the mass. If necessary, filter the water layer and washings and collect in a calibrated flask. Reserve for further work.

Waxes. Dissolve the waxy mass, including any on the filter paper, in 200 ml. of hot 95 per cent alcohol (S. D. 3A will do). Carefully boil the mixture to ensure complete disintegration, transfer to a 250 ml. centrifuge bottle, and cool to room temperature. Centrifuge until a clear upper layer is formed. If a semigel structure forms, add a large excess of alcohol and use two centrifuge bottles. Decant off the clear liquid, add more alcohol to the bottle, and bring to the boil in a water bath. Shake thoroughly, cool, and centrifuge as before. Decant and repeat the whole operation at least once or until the cold alcohol extract is colorless. Combine the alcohol extracts and reserve.

Add 100 ml. of trichloroethylene to the residue in the centrifuge bottle and bring to a boil in a water bath. Shake well, and if any insoluble matter is present, centrifuge while hot. Decant and wash the insoluble matter with small portions of hot trichloroethylene until the solvent is colorless, centrifuging if necessary. Evaporate the trichloroethylene extract and washings. It may be necessary to keep the extracted waxes in the oven at 105° to 110° for several hours before all the solvent is gone. After heating to constant weight, obtain such constants on the waxes as melting point, acid value, saponification value, and aniline point. Observe the odor on burning. Carnauba wax is most commonly employed but is frequently modified, for economy, with petroleum or mineral waxes, vegetable waxes such as candelilla and ouricury, and cumar-indene resins and polymerized pine-type resins such as piccolyte. These will all dissolve in hot trichloroethylene. If the character of this extract indicates a synthetic resin as well as waxes, boil it with 100 ml. of 95 per cent alcohol and centrifuge hot. Repeat at least twice more, being sure to keep the alcohol very hot. Cumar and piccolyte are insoluble in hot alcohol, whereas vegetable waxes are soluble in hot alcohol on repeated extraction.

Small portions of some natural resins are soluble in trichloroethylene, and about 5 per cent of the natural mixture of waxes in carnauba wax is soluble in cold alcohol. Such small sources of error tend to counteract each other, but this error should be taken into account in interpreting results.

Resins and Fatty Acids. These are in the alcohol-soluble portion. There are no simple methods for separating the resins and the soap fatty acids. Usually the soap in the original compound has been partially formed from the acidic portion of the resin and partially from added fatty acids or added as soap itself.

Dilute the combined alcohol extracts to 500 ml.; on one aliquot obtain total solids and on other aliquots determine the acid value and saponification value. Test for rosin on the solids by the Liebermann-Storch reaction. Useful information as to colors obtained with various natural resins using the Liebermann-Storch reaction have been published (*3*). Notice the odor on burning and identify as rosin, copal, dammar, or other type of resin.

Any insoluble matter obtained after extracting the trichloroethylene-soluble is usually resinous and can be expected to be an alcohol-insoluble fraction of the original resin used. It may be identified as was the alcohol-soluble portion.

Amines. Soaps of ammonia, mono-, di-, and triethanolamine, morpholine, aminomethylpropanol, and other amines are all used in water-base waxes as dispersing agents. Frequently only a guess can be made as to which is present. Experience indicates that some give off ammonia on heating with sodium hydroxide more readily than do others. With mixtures, such as are at times employed, the difficulty increases.

Dilute the aqueous portion, reserved after acidification, to 250 ml. and obtain total solids on an aliquot. The rate at which the solids lose weight is somewhat indicative of the type of amine present. Keep one aliquot in the oven, after evaporating off the bulk of the water on the steam bath for just sufficient time to be sure that all the water is removed. Cool and weigh. Deduct the weight of chloride equivalent to any fixed alkali found in the ash. The residual weight will be that of the hydrochloride of the amine or mixture of amines.

On another aliquot, determine total nitrogen by the Kjeldahl method, calculate to the suspected amine, and further calculate to the hydrochloride of that amine. Thus, by trial and error calculations, a fair estimate of the type of amine, and also its amount, may usually be obtained. The interpretation of results so obtained in the shape of a formula requires considerable practical experience and knowledge both of the chemical and physical properties of the waxes, resins, and amines, and of the physical properties they impart to the finished product.

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Polarographic Analysis of Copper and Zinc in Brass Plate

A Rapid Control Method

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The existing methods of brass plate analysis for control purposes are either slow or inaccurate. A method is described whereby brass plate consisting of copper and zinc only may be analyzed by means of a polarograph if the base metal is iron or steel.

The brass plate is dissolved in ammoniacal ammonium persulfate. This solution is added to a supporting electrolyte solution containing ammonium hydroxide, ammonium chloride, sodium sulfite, and gelatin and the polarogram is read after oxygen and persulfate have been reduced by the sulfite. Since only the ratio of diffusion currents of zinc and copper ions is measured instead of exact diffusion currents of the individual ions, exact temperature control is unnecessary, less care is required in measuring solutions, and the results are independent of the capillary used.

The entire analysis requires an elapsed time of 20 minutes, but only about 10 minutes of the operator's time. Thus, six analyses can be made per hour in routine work. The method may be depended upon to give results within ±1 per cent of the copper percentage in rapid control work and within ±0.5 per cent when extra precautions are taken.

THE existing methods for the analysis of copper and zinc in brass plate are not particularly suitable for rapid routine control. The most rapid methods, those involving separation with hydrogen sulfide, require 20 to 30 minutes of the analyst's time and are not capable of very high accuracy or reproducibility under production conditions. Methods involving electrolytic separations are more accurate and require less of the analyst's time. However, the total time required for the completion of the analysis is frequently so great that production lines may be held up if analysis is necessary for the release of plated articles.

It was thought that the polarograph would offer an excellent means for the determination of the composition of pure copper-zinc brass plate if suitable conditions could be devised. The method of Hohn (*1*) for brass analysis and the method of Kraus and Novak, cited by Kolthoff and Lingane (*3*), for copper and zinc in zinc ores offered proof that a polarographic method would be practical for brass plate if it could be made more adaptable to rapid routine control.

With these facts in mind, a polarographic method was developed for the analysis of copper-zinc brass plated on iron or steel. The method makes use of the "pilot ion" system, in that the ratio of zinc to copper is determined rather than the actual amounts of each ion present. Thus, exact temperature control is not needed, less care is required in measuring solutions, and the results are independent of the capillary used. The entire analysis requires a total time of about 20 minutes and only 10 minutes of the operator's time. It may be depended upon to give results within ±1.0 per cent of the true copper percentage in rapid control work, and within ±0.5 per cent when extra precautions are taken.

Solid brass samples are dissolved somewhat slowly by the reagent used in this investigation. However, the rate of solution may be increased to make the method applicable to solid brass by agitation, the use of a more concentrated solution of the reagent, and the use of small and finely divided samples. It should be possible to measure the zinc to copper ratio in the absence of interfering metals and to calculate the actual per cent of copper in special cases when the quantity of metal present other than copper and zinc is known.

Apparatus

The polarographic apparatus used in these studies was the Fisher Elecdropode, a manually operated instrument equipped with an adjustable galvanometer sensitivity control and a residual current compensating device. The measurements were made with the quiet mercury pool as the anode. In order to refer the potentials to the saturated calomel electrode (S. C. E.) it was necessary to measure the pool to calomel electrode potential in a separate operation and correct the former readings with this value. The range of measurement of pool to calomel electrode potential is limited to ±0.2 volt. The conditions chosen for the analyses were such that the calomel-pool potentials were of the order +0.19 volt, and were somewhat variable. In those cases in which the potential was greater than 0.2 volt it was possible to approximate the value with sufficient accuracy by extrapolation.

The capillaries used in this work had initial drop times of approximately 3 seconds. The mercury used in the pool was purified by drawing air through it under 10 per cent nitric acid, washing with distilled water, drying, and "pinholing", while that used in the capillary was further cleaned by vacuum distillation.

Solutions

Standard Brass Solutions. Solutions of the desired composition were prepared by mixing measured volumes of standard copper sulfate and zinc sulfate solutions. The copper solution was standardized electrolytically. The zinc solution was standardized with potassium ferrocyanide solution which was standardized against a standard solution of zinc prepared from the pure metal. Diphenylbenzidine was used as an internal indicator in the zinc standardization. The solutions used in these experiments contained about 4.0 grams of copper and 1.7 grams of zinc per liter. The standard solutions for polarographic use were made from salts rather than from the pure metals because of the undesirable effect of nitrate on the polarogram.

Standard Ammonium Persulfate Solution. This solution is 3.75 *N* in ammonium hydroxide and is made up to contain 17.5 grams of c. p. ammonium persulfate per 100 ml. of solution. Dilution of this solution according to the procedure for standardization results in a solution containing the amount of persulfate that is present when 15 mg. of 70–30 brass plate are stripped with 5 ml. of stripping solution.

Stripping Solution. The solution contains 80 grams of ammonium persulfate per liter of solution and is 1.5 *N* in ammonium hydroxide. c. p. grade salt was used in these experiments, but in

Table I. Analysis of Synthetically Prepared Samples

Sample	Temperature	Copper Polarographically	Copper Known	Copper Error
	° C.	%	%	%
1	26.7	54.0	54.30	−0.30
2	26.1	60.1	60.25	−0.15
3	26.6	69.3	69.45	−0.15
4	25.8	65.8	65.80	+0.00
5	26.5	73.5	73.47	+0.03

Temperature of standardization: 25.5° C.

later work the several batches of technical grade material that were tested were found to be free of interfering substances.

Supporting Electrolyte. This solution must be free from copper and zinc. It has the following composition:

1.0 molar in c. p. ammonium chloride
1.5 molar in c. p. ammonium hydroxide
0.54 molar in c. p. sodium sulfate
0.67 gram of gelatin per liter

Procedure

Standardization Procedure. Pipet 3.0 ml. of standard brass solution into a small beaker. Add 2.0 ml. of standard ammonium persulfate solution and 15 ml. of supporting electrolyte solution. Mix and allow to stand covered for 10 minutes to effect complete reduction of persulfate and of oxygen. Cool to within 0.5° C. of the prevailing room temperature, place in the electrolysis cup containing the pool mercury, and measure the potential of the pool with reference to the saturated calomel electrode. Note the temperature of the solution and record the currents (galvanometer deflections) at the following voltages (*vs.* S. C. E.): A at −0.36 volt; B at −1.03 volts; D at −1.605 volts. If the temperature has changed more than 0.2° C. during the run, repeat the readings. For very accurate work a fourth current, C, at −1.305 volts (*vs.* S. C. E.) must be determined. −0.36 is the only point at which the voltage must be set accurately, since the other points occur in flat regions of the polarogram. Variations of 25 millivolts usually have no effect on the current at the other voltages. The above voltages were chosen at the flattest portions of the current-voltage curve.

Brass Plate Analysis. Strip the brass plate from a desired area of the sample by flowing a given quantity of stripping solution over the surface, using a pipet fitted with a rubber bulb, until the brass has been entirely removed. Do not allow solution to come in contact with rubber. The area stripped should be such that 15 mg. of brass are present in a 5-ml. portion of the stripping solution, although in routine work this may vary from 5 to about 25 mg. per 5-ml. portion. The warmer the sample the more rapid the stripping, but it should not be hot enough to volatilize an appreciable amount of ammonia.

Measure 5 ml. of the brass solution into a beaker, add 15 ml. of supporting electrolyte solution, cover, and let stand for 10 minutes. Cool to within 0.5° C. of the room temperature and continue exactly as in the standardization. The proper sensitivity range of the galvanometer to use may be determined from the magnitude of the current at −0.36 volt (*vs.* S. C. E.).

Experimental Results

A series of samples, made synthetically from measured portions of the standard copper sulfate and zinc sulfate solutions described above, were prepared to contain the same concentrations of stripping solution and approximately the same amounts of ferric hydroxide as are found in samples obtained from plated articles. They were then analyzed by one of the authors without knowledge of their composition. The results, obtained by the accurate method of calculation described below, are compared in Table I with the known values.

A second series of samples was taken from brass plate in production. A portion of each sample was analyzed with the Elecdropode, and the results were calculated by the accurate method. The remainder of each sample was then analyzed for copper electrolytically. The solution after electrolysis was evaporated to sulfuric acid fumes, the small amount of iron was removed with ammonium hydroxide, and the zinc was determined with standard potassium ferrocyanide, using diphenylbenzidine as an internal indicator. The data obtained by the two methods are compared in Table II.

Calculations

In calculating the diffusion currents from a polarogram it is usually necessary to take into consideration two factors, the residual current due to the supporting electrolyte and the change in the current with potential above and below the electrocapillary zero potential.

Correction for the former was found to be unnecessary, at least for quantities of brass greater than 5 mg., because of the very low and almost linear residual current-voltage curve of the supporting electrolyte.

In order to correct for the change in current with increase in potential it is necessary to measure the values of $m^{2/3}t^{1/6}$ at the potentials used and to correct the diffusion currents as described by Kolthoff (*2*). The mass of the mercury dropping per second, m, and the drop time in seconds, t, were measured at the four potentials employed in the procedure, using a typical brass plate analysis solution for the electrolyte. The electrocapillary zero potential appears to be between −0.40 and −0.45 volt (*vs.* S. C. E.). The values of $m^{2/3}t^{1/6}$ at −0.36, −1.03, −1.305, and −1.605 volt (*vs.* S. C. E.) were 2.035, 2.009, 1.982, and 1.939 mg.$^{2/3}$ sec.$^{-1/2}$, respectively. The correct diffusion currents of copper and zinc are calculated as follows:

$$i_d\,(\mathrm{Cu}) = B - (2.009/2.035)\,A = B - 0.987\,A$$
$$i_d\,(\mathrm{Zn}) = D - (1.939/1.982)\,C = D - 0.978\,C$$

where A, B, C, and D are the currents at the above-mentioned potentials as previously defined. The above method of calculating the diffusion currents is referred to in this paper as the accurate method of calculation.

Table II. Comparison of Polarographic and Chemical Analysis of Brass Plate

Sample	Temperature	Copper Polarographically	Copper Chemically	Difference
	° C.	%	%	%
1	25.1	69.5	69.10	+0.40
2	25.0	76.5	76.50	+0.00
3	24.5	69.3	69.45	−0.15
4	23.8	68.9	68.80	+0.10
5	23.8	72.8	72.50	+0.30
6	24.1	71.1	71.05	+0.05

Temperature of standardization: 1 and 2 = 24.8° C., 3 to 6 = 24.1° C.

In order to decrease the time for analysis and calculation in routine work it was found permissible to measure only diffusion currents A, B, and D and to make an empirical correction of the final copper percentage. For this method, the diffusion currents are defined as follows:

$$i_d\,(\mathrm{Cu}) = B - A$$
$$i_d\,(\mathrm{Zn}) = D - B$$

In either case the calculation of per cent copper is as follows:

The ratio of the diffusion currents of the analyzed sample is

$$R_x = i_d\,(\mathrm{Zn})/i_d\,(\mathrm{Cu})$$

In order to obtain the weight ratio, R_w, of zinc to copper in the analyzed sample, divide R_x by the corresponding diffusion current ratio for the standard brass solution and multiply by the weight ratio of zinc to copper in the standard brass solution. Then,

$$\%\ \mathrm{Cu} = 100/(1 + R_w)$$

If the empirical correction method is to be used when the standard solution is 70 per cent copper, add 0.1 per cent to the

calculated value for each 1 per cent below 70 per cent and subtract 0.1 per cent for each 1 per cent above 70 per cent. The limits within which this method has been applied are 60 to 80 per cent copper. No data are available using other standard solutions.

It is frequently desirable to know the approximate average thickness of a brass plate. If the brass is 70 ± 5 per cent copper and the temperature of the analysis is within 1° C. of that of the standardization, this can be done by comparing the sum of the diffusion currents of the sample and the standard. It is thus possible to calculate the quantity of brass in the analyzed sample if the quantity in the standard solution is known, and to calculate the thickness of the brass plate to within 5 per cent of the true average value without resorting to carefully controlled conditions.

Discussion

The problem of selecting the best supporting electrolyte solution was simplified by a limited choice of methods of stripping the plate from the base metal. The three common reagents used for this purpose are nitric acid, ammoniacal ammonium trichloroacetate, and ammoniacal ammonium persulfate. The use of nitric acid is not practicable because of the large amounts of iron that may be dissolved. Trichloroacetate solution produces cathodic waves in ammoniacal solution and therefore cannot be used. Persulfate also produces a cathodic wave, but this ion can be reduced successfully with sodium sulfite which simultaneously removes the oxygen. Experiment showed that the final solution should be at least 1.5 molar in ammonium hydroxide and 0.75 molar in ammonium salts, and should contain about 0.5 gram of gelatin per liter; 0.4 molar sodium sulfite provides a safe excess of reducing agent. Higher concentrations of ammonium hydroxide and ammonium chloride can be used and appear to make the readings less sensitive to slight changes in supporting electrolyte concentrations, but the above concentrations proved to be satisfactory. Lower gelatin concentrations have a tendency to give erratic results because of failure to remove maxima completely and because of more pronounced variations in the diffusion current ratio with slight differences in the gelatin content. Lower sulfite concentrations increase the time of standing required before the polarogram can be obtained and increase the error due to variations in final sulfite concentrations.

The effect of temperature on the ratio of the diffusion current of zinc to that of copper is small compared to its effect upon the individual diffusion currents of zinc and copper. This ratio increases only about 0.25 per cent per degree change in temperature, indicating a slightly greater temperature coefficient of diffusion current of the zinc ammonia ion than of the copper ammonia ion. Thus a temperature difference of 2° C. between an analysis and the standardization will affect the ratio by 0.5 per cent which is within the limits of accuracy of the measurements. The temperature must, however, be maintained constant to 0.2° C. during the current-voltage readings of any given analysis or standardization because of variation with temperature of one diffusion current without an equivalent change in the other. This is comparatively simple, since the time required for a given set of readings is only about 2 minutes.

The current-voltage curves are normal in form, showing a portion of one wave and a full second wave for copper and a single wave for zinc. They also show the decrease in current due to decrease in drop time with increase in potential above the electrocapillary zero potential. The half-wave potentials are very close to those given by Kolthoff and Lingane (*4*), −0.48 (*vs.* S. C. E.) volt for cuprous ion to copper and −1.43 volts (*vs.* S. C. E.) for zinc ion to zinc, in supporting electrolytes high in ammonium hydroxide and ammonium chloride.

Fresh standard ammonium persulfate solution should be made each week before standardization. The supporting electrolyte solution will keep indefinitely out of contact with air. The stripping solution should be kept in a stoppered bottle and may deteriorate after 1 or 2 weeks. None of the solutions should be allowed to come in contact with rubber before or during use. Koroseal tubing has been found satisfactory for dispensing the supporting electrolyte solution, but no tests have been made with any tubing for dispensing the stripping solution.

If the sample is to be rinsed, only stripping solution should be used for this operation. Rinsing is not essential in routine work. All dilutions of the stripped "brass" solution must be made with stripping solution. Experience will enable the operator to decide whether such dilution is necessary to keep the solution within the specified concentration limits. If the solution must be more concentrated, more brass can be stripped with the solution which contains the previously stripped brass.

Because the action of persulfate on mercury produces ions which affect the residual current, the reduction of the persulfate with the sulfite must be done before the solution comes in contact with mercury.

The action of ammonium persulfate on the iron from which the brass has been stripped will produce "rust" spots, particularly when the sample is hot. A normal amount of this reaction is permissible, but an excessive amount may decompose too much persulfate and thus change the concentration of sulfite ion in the final solution.

A number of elements such as nickel, cobalt, manganese, and cadmium, which are reduced under the conditions of this determination, interfere with the analysis. Small amounts of lead do not affect the polarograms, while the insolubility of the hydroxide prevents the interference of iron. The presence of the precipitate of iron hydroxide in the electrolysis solution does not measurably affect the polarogram.

Since the success of the method depends on the maintenance of a constant supporting electrolyte concentration, a large excess of sodium sulfite is used. This leaves a relatively constant amount of sulfite ion in the final electrolysis solution, regardless of slight variations in the quantity of persulfate used up in the stripping process. Thus it is permissible to use only one standardization for a range of samples containing from 5 to 25 mg. of brass per 5 ml. of solution without introducing appreciable error into routine analysis. The difference between the ratio of the diffusion currents which would be obtained when the minimum (5 mg.) and the maximum (25 mg.) of brass are present in 5 ml. of solution has been found to be about 1.5 per cent. When this value is applied to the calculation of per cent copper in a 70-30 brass, it represents an error of about 0.5 per cent copper. The mean value between these limits is found when the solution contains approximately 15 mg. of brass in 5 ml. Therefore, if the standardization conditions simulate those existing when 15 mg. of brass are stripped by 5 ml. of stripping solution, the maximum error which can be attributed to this source will be only ±0.25 per cent copper. In more accurate work, empirical corrections or calibration of the instrument with persulfate concentrations corresponding to various brass concentrations will reduce this error.

The temperature effect on the ratio of diffusion currents has been previously noted as causing an error of about 0.25 per cent per degree. This causes an error of about 0.1 per cent per degree in the copper percentage in 70-30 brass. This error may be eliminated in more accurate work by the

use of more exact temperature control or of temperature coefficient corrections.

The reproducibility of the method was determined by repeated standardizations with varying "brass" concentrations using different galvanometer sensitivities, with and without compensation for the residual current of the copper wave. The diffusion current ratio was found to be reproducible to within ±0.5 per cent average deviation from the mean if the galvanometer has a linear response and if the temperature is constant within 1°. The maximum probable error is about 1 per cent of the ratio. This represents an error of about ±0.3 per cent copper in 70-30 brass, and can be safely said to represent the maximum error of the method using carefully controlled conditions and the accurate method of calculation.

The error introduced by using the rapid empirical method of correcting the copper percentages is very small. In 28 determinations the maximum difference between values obtained by this method and the accurate method of calculation was 0.3 per cent copper and the average difference was 0.1 per cent copper.

The maximum probable error of the routine method of analysis in which the temperature is controlled only to ±2° C., one standardization is used for all quantities of brass from 5 to 25 mg. per 5 ml. of solution, and the rapid empirical method of calculation is used, can be found from the above discussion to be about ±1 per cent copper. The average error will be considerably less than this value.

Summary

A polarographic method is described for the determination of copper and zinc in brass plate on iron or steel, which is capable of determining the copper percentage to within ±1 per cent in routine analysis and within 0.5 per cent under carefully controlled conditions.

The total time for a single analysis is approximately 20 minutes, and since the attention of the operator is required for less than 10 minutes, about six analyses can be made per hour.

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Water Thermoregulator

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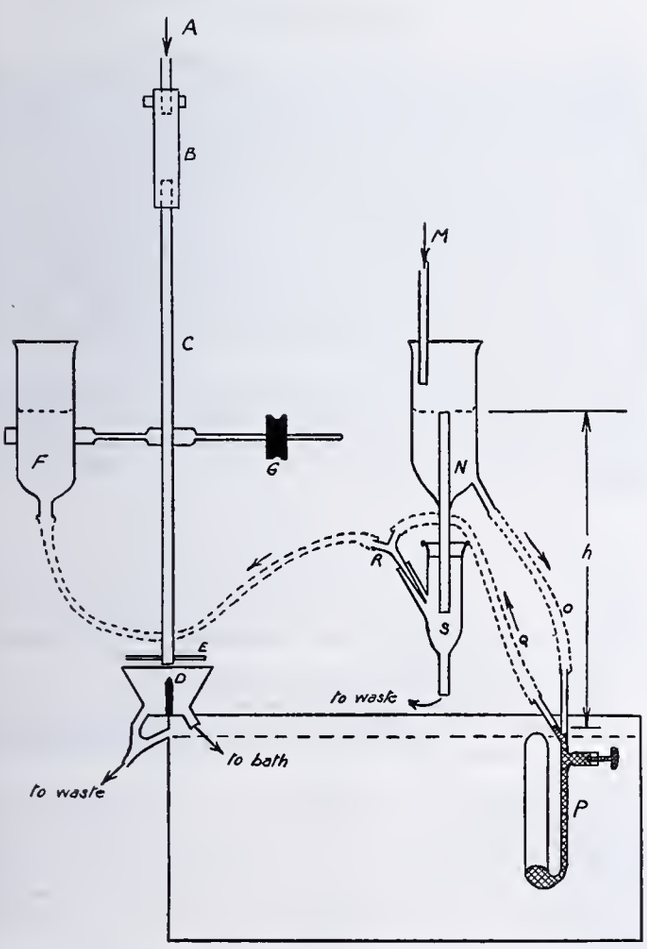

Figure 1

An inexpensive, easily portable thermoregulator, capable of maintaining a variation of ±0.5° C. in bath temperature, is quickly constructed from a branched-tube regulator using a mercury valve. No electricity is used, power being obtained directly from the feed water.

As shown in Figure 1, the bath was used at 20° C., with warm water entering *A* to keep it up to temperature. Above 20° C. an external heater is to be preferred, with cold water entering at *A* controlling the excess quantity of heat supplied.

The regulator consists of two parts: the pendulum group and the valve group.

The pendulum group consists of a tube, *C*, of 7-mm. bore, about 45 cm. (18 inches) long, suspended by a piece of rubber tubing, *B*, firmly clamped at its upper end. The amount of swing allowed the pendulum is regulated by the slotted guide plate, *E*. A tube, *F*, holding at least 10 ml. of water, is held on an arm at right angles to the vertical tube, *C*, but free to move vertically up and down it. A weight, *G*, counterbalances the weight of *F*.

The valve group consists of a toluene-mercury regulator, *P*, with a branched tube serving as a valve. A vessel holding at least 75 ml. of toluene is recommended. The adjusting device consists of a large-diameter knurled setscrew entering a 7-mm. glass side arm. The vertical tube and side arm are made of 3-mm. bore tubing. *N* is a constant-level device, while *R* is a bleeder emitting water to the drain at approximately 30 ml. per minute.

The action is as follows:

A small amount of water enters the valve group at *M*, flowing into the constant-level tube, *N*. Any excess is led to waste. Water flows down tube *O* into the vertical arm of the regulator and up the inclined arm through tube *Q* to fill vessel *F*. The weight of *F* is now great enough to force tube *C* over and deliver water into the bath. As the temperature rises the mercury assumes the shape as shown in the diagram and seals off tube *Q*, preventing water from reaching *F*. Bleeder *R*, though operating continuously, is now able to empty *F* in a few seconds and allows tube *C* to swing back over the baffle. When the temperature drops the mercury falls, allowing the water to reënter *Q*, and the cycle is repeated.

No cutting off of the mercury column takes place when the water leaves by way of the inclined tube. A small head, *h*, of water is to be preferred. Good results were obtained when a head of 15 to 20 cm. (6 to 8 inches) was used. A temperature variation of ±0.5° C. was easily maintained in 6-day runs.

Calibration of a Photoelectric Colorimeter for the Determination of Chlorophyll

Relation between Spectra of Standards and Accuracy of Analytical Results

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CHLOROPHYLLS *a* and *b*, because of their characteristic absorption spectra, comprise a system which is eminently suitable for the application of photoelectric colorimetric and spectrophotometric methods of analysis. An accurate and relatively simple spectrophotometric method for the determination of the individual components, as well as total chlorophyll, based on the fundamental absorption spectra of chlorophylls *a* and *b* is available (*1*, *2*). The situation with regard to the photoelectric colorimetric evaluation of total chlorophyll, however, is less satisfactory, primarily owing to the uncertainties encountered in obtaining, preparing, storing, or using chlorophyll preparations and the sensitivity of the analytical values to the spectrum of the standard used for the calibration of the instrument.

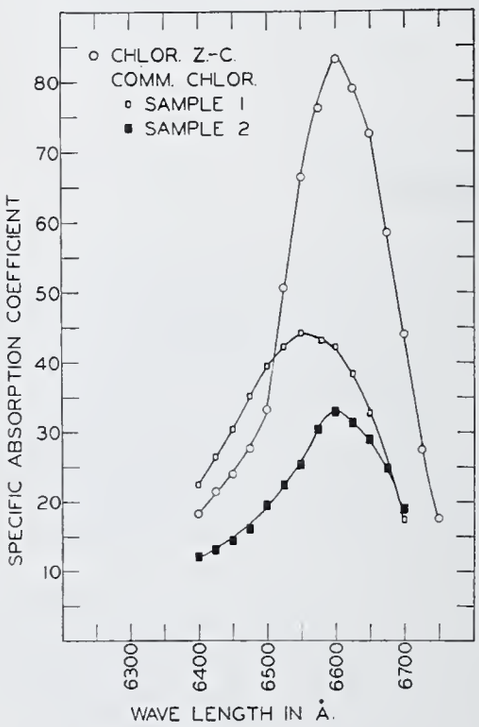

FIGURE 1. ABSORPTION SPECTRA OF CHLOROPHYLL PREPARATIONS IN DIETHYL ETHER SOLUTION

In the spectrophotometric method as described by Comar and Zscheile (*2*) and Comar (*1*) the pigments must be transferred from the original acetone extract to diethyl ether solution and the light-absorption readings made on an optical instrument capable of isolating a spectral region of about 30 Å. near wave length 6600 Å. with negligible stray radiation. The necessary washing of the ether solution is rather time-consuming and high quality optical equipment is required to fulfill the above conditions. In the many instances where it is sufficient to evaluate only total chlorophyll the determination may be greatly shortened and simplified by making the measurements directly on the acetone extract and using a photoelectric colorimeter and calibration curve instead of the more complicated and often less available spectrophotometer. Petering, Wolman, and Hibbard (*5*) determined chlorophyll in this way, using 5X chlorophyll from American Chlorophyll, Inc., for the calibration standard. However, as in any absolute colorimetric method, accurate results will be obtained only if the absorption of the standard is identical with that of the substance under consideration as measured in the unknown solution. This is of particular importance in the case of chlorophyll, because of the instability of the isolated pigment, the sensitivity of its spectrum to the formation of degradation products, and the variations in the chlorophyll *a* to *b* ratio which occur as a result of preparative procedure.

The work presented here shows the relation between the absorption spectra of the standard chlorophyll and the accuracy of the analytical results and presents a means of calibration which avoids the difficulties incurred by the use of chlorophyll standards in photoelectric colorimetry.

Experimental

Three samples of chlorophyll (*a* + *b*) were used: (1) a research grade of chlorophyll purchased from a domestic concern (designated in this paper as commercial chlorophyll, Sample 1). According to the manufacturer it was prepared in June, 1942, and stored in a cool dark place until February, 1943, at which time the measurements were made. (2) A research grade of chlorophyll purchased several years ago from the same company and stored for the most part at 5° C. and in the dark during that period (designated as commercial chlorophyll, Sample 2). (3) Chlorophyll prepared in this laboratory by the method of Zscheile and Comar (*7*) in August, 1941. This sample had been dried at the time of preparation and was stored at 5° C. in the dark until used in the work reported here, October, 1942 (designated as chlorophyll Z.-C.).

The absorption spectra of these chlorophylls in diethyl ether solution are presented in Figure 1. They were determined by means of a Cenco-Sheard spectrophotelometer under instrumental conditions as described by Comar (*1*). Beer's law is used in the form:

$$\alpha = \frac{\log_{10} \frac{I_0}{I}}{cl} \qquad (1)$$

where α = specific absorption coefficient (liters per gram-centimeter)
c = concentration (grams per liter)
l = thickness of solution layer
I_0 = intensity of light transmitted by solvent-filled cell
I = intensity of light transmitted by solution-filled cell

For the colorimetric evaluations a Cenco photelometer was used with a combination of Corning H. R. light filters Nos. 243 and 396 of standard thickness. The instrumental details were as described by Petering, Wolman, and Hibbard (*5*). The calibration curves for the three chlorophyll samples in 85 per cent acetone solution are represented by the upper three curves of Figure 2.

The lowest curve in Figure 2 (chlorophyll-plant extract) was obtained in the following way. A sample of fresh leaf tissue

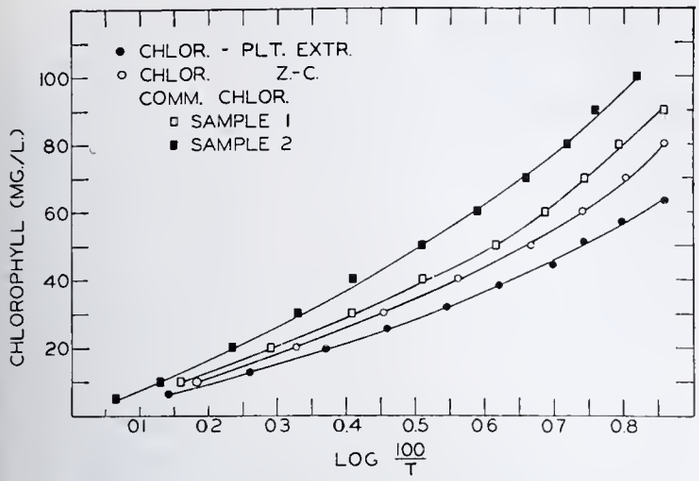

FIGURE 2. COMPARISON OF CALIBRATION CURVES OBTAINED BY USING DIFFERENT CHLOROPHYLL STANDARDS

TABLE I. DATA FOR PLANT EXTRACT CALIBRATION CURVE

Dilution: Stock solution	Dilution: 85% acetone	Chlorophyll Content	Photelometer Reading, % Transmission	$\log_{10} \frac{100}{T}$
Ml.	*Ml.*	*Mg./l.*		
10	0	63.7	13.8	0.860
9	1	57.3	15.9	0.798
8	2	51.0	18.0	0.744
7	3	44.6	20.0	0.699
6	4	38.2	23.9	0.622
5	5	31.9	28.5	0.545
4	6	25.5	34.7	0.459
3	7	19.1	43.0	0.366
2	8	12.7	54.9	0.260
1	9	6.4	72.0	0.142

was disintegrated in a Waring Blendor in 85 per cent acetone to which a small amount of calcium carbonate had been added. The macerate was filtered through paper by suction and the filtrate made to volume with 85 per cent acetone. An aliquot was transferred to ether and the absolute chlorophyll content determined spectrophotometrically as described by Comar (*1*). A series of dilutions was then made from the stock acetone solution and the light transmission of each of these dilutions was measured on the photelometer as described by Petering, Wolman, and Hibbard (*5*). The chlorophyll content of each of the above solutions was calculated from the value obtained for the stock solution by the spectrophotometric method and the calibration curve was then constructed. The following experiment will serve to illustrate the calibration procedure.

A 4.00-gram sample of fresh leaf tissue from Norway maple was extracted with 85 per cent acetone as described and made to 250 ml. Following the Comar method (*1*), a 25-ml. aliquot of the acetone solution was transferred to ether, and after washing made to 100 ml. It was then necessary to dilute this solution four times for the spectrophotometric measurements in order to bring the $\log_{10} \frac{I_0}{I}$ values into the accurate range. This yielded the following values, using a 2-cm. absorption cell:

$$\log_{10} \frac{I_0}{I} \text{ at } 6600 \text{ Å.} = 0.565$$

$$\log_{10} \frac{I_0}{I} \text{ at } 6425 \text{ Å.} = 0.234$$

From these values it is calculated that the chlorophyll concentration of the solution measured with the spectrophotometer was 3.98 mg. per liter [see (*1*) for method of calculation]. Since there was an over-all dilution of 16, the chlorophyll content of the acetone solution was (3.98) (16) = 63.7 mg. per liter. The original acetone solution was then diluted as shown in Table I and the measurements were made with the photelometer.

The values of chlorophyll content in Table I are calculated from the dilution factor and the concentration of the stock solution as determined spectrophotometrically, and the calibration curve is then constructed by plotting the per cent transmission, or $\log_{10} \frac{100}{T}$ value, against chlorophyll content. A calibration curve obtained using tomato leaves was identical with that reported above.

The following procedure was used for the analyses given in Table II:

A weighed sample of the fresh leaf tissue was disintegrated as described above and the 85 per cent acetone solution made to volume. An aliquot of this solution was transferred to ether and analyzed spectrophotometrically by the Comar method (*1*). Another aliquot of the original acetone solution was analyzed colorimetrically by the Petering, Wolman, and Hibbard method (*5*), using the filter instrument and each of the calibration curves.

Discussion

Numerous workers (*3*, *4*, *6*, *7*) have discussed the disagreements in the reported spectroscopic values for the chlorophylls and the factors involved in chlorophyll degradation. It is to be emphasized that chlorophyll is not stable, and tends to form pheophytin as one of the first products of decomposition. As a rule the chlorophyll degradation products absorb less in the region near 6600 Å. than the pure pigment; therefore any such mixture will have an absorption in this region lower than that of pure chlorophyll. This is considered to account for the differences shown in Figure 1. Both commercial samples of chlorophyll exhibited the increased typical pheophytin absorption in the region near 5100 Å. which indicated that the lowered absorption was not due merely to the presence of inert impurities. Commercial chlorophyll, Sample 1, was unusual in that the red peak position was shifted 50 Å. towards the shorter wave lengths as compared to the normal chlorophyll. Zscheile and Comar (*7*) found that research grades of chlorophyll *a* and chloro-

TABLE II. CHLOROPHYLL ANALYSES

(Results by the Spectrophotometric Method Compared with Those by the Photoelectric Colorimetric Method Using Different Calibration Standards with the Latter[a])

Plant Leaf Material	Spectrophotometric Method, % Chlorophyll *a*	Photoelectric Colorimeter Calibrated with: Chlorophyll in plant extract	Chlorophyll by Z.-C. method	Commercial chlorophyll, Sample 1	Commercial chlorophyll Sample 2
		Mg. of total chlorophyll per gram of leaf tissue			
Norway maple	68.5	3.98	4.12	4.88	5.38	7.00
Muskmelon	68.4	1.75	1.77	2.12	2.41	3.20
Alfalfa	70.3	3.07	2.95	3.52	3.99	5.23
Crimean linden	67.8	2.83	2.88	3.48	3.82	5.00
Tomato	69.8	1.69	1.69	2.03	2.32	3.01
Lima bean	70.7	2.18	2.10	2.47	2.88	3.70
Watermelon	70.9	1.43	1.57	1.87	2.13	2.78
Peanut	70.5	2.09	1.99	2.40	2.70	3.60
Bush string bean	69.8	1.84	1.81	2.06	2.56	3.08
Pepper	69.8	2.03	2.01	2.29	2.76	3.39
Oats	72.5	2.02	1.95	2.35	2.67	3.46
Wheat	73.2	2.02	1.98	2.39	2.70	3.53
Broccoli	70.0	2.76	2.60	3.14	3.52	4.67
Spinach	71.9	1.25	1.20	1.45	1.65	2.13
Beets	72.2	1.10	1.07	1.31	1.56	1.90
Carrot	71.1	2.09	2.02	2.44	2.77	3.62
Blue grass	70.0	2.48	2.46	2.94	3.33	4.33
Sunflower	70.8	1.89	1.80	2.16	2.48	3.26
Rhubarb	69.2	0.79	0.79	0.95	1.08	1.40
Black locust	69.3	3.15	2.92	3.57	4.02	5.27
Hollyhock	72.3	2.16	2.07	2.50	2.84	3.73
Peach	71.2	2.19	2.41	2.90	3.30	4.36
Clover	71.8	2.74	2.68	3.19	3.57	4.64
Burdock	70.5	2.11	2.02	2.42	2.79	3.67

[a] Description of standards given under Experimental.

phyll *b* purchased from a company in Switzerland showed the presence of significant amounts of pheophytin.

Another factor affecting the height of the absorption curve is the relative amounts of chlorophyll *a* and *b* present. It may be observed from the spectra of chlorophyll *a* and *b* (*1*) that the higher the ratio of *a* to *b* the greater will be the absorption in the region near 6600 Å. This factor is not important from the point of view of the ratio of *a* to *b* in the usual plant material to be analyzed, since this ratio is remarkably constant. Table II indicates that the variation in percentage of chlorophyll *a* for the 24 plants studied is not great enough to cause significant errors in the determinations as described here. However, in the isolated chlorophyll (*a* + *b*) used as the calibration standard this ratio may be altered by the preparative procedure. For instance, in the Zscheile-Comar method of preparation (*7*) it is necessary to use a methanol wash to eliminate xanthophylls and this preferentially removes chlorophyll *b* which is more soluble than chlorophyll *a* in methanol. Thus the Zscheile-Comar sample represented in Figure 1 contained about 84 per cent chlorophyll *a* instead of the 68 to 72 per cent found in the usual leaf tissue.

As would be expected, the spread shown by the calibration curves, Figure 2, parallels in general the differences as found in the spectra of the chlorophyll standards, Figure 1. Numerical relations between the calibration curve and the height of the absorption peaks cannot be easily calculated, however, since with the colorimeter the integrated absorption over the entire spectral region isolated by the filter system must be considered.

It follows that differences in the absorption of the standards that carry through into the calibration curves will be reflected in the analytical values obtained. This is evident from the results presented in Table II. Satisfactory agreement is obtained between the spectrophotometric method and the colorimetric method employing the plant extract calibration curve. Out of the 24 plants studied, only the results from peach leaves showed significant disagreement. The cause of the discrepancy is not evident and the necessity for further study of this particular plant material is indicated.

In the case of all results where the calibration curves from the chlorophyll standards were employed, the deviations from the spectrophotometric values were considerable and were of the same order of magnitude for any given standard. The values obtained using Commercial Chlorophyll, Sample 1, were all about 35 per cent higher than the corresponding spectrophotometric values, those with commercial chlorophyll, Sample 2, were about 75 per cent higher, and those with the chlorophyll prepared in this laboratory about 18 per cent higher. In all cases the precision was excellent and recovery of the added standard in each instance would have been accurate; however, this cannot be considered as a criterion of how nearly the analytical results will approach the absolute chlorophyll values.

It is not easy, then, to obtain chlorophyll sufficiently pure for use as a calibration standard. Commercial samples studied have not been found reliable and are actually unobtainable at the present time. The isolation of chlorophyll in the laboratory is difficult for inexperienced workers, and unless extreme precautions are taken will not always yield an uncontaminated product; Zscheile and Comar (*7*) have shown that drying chlorophyll tends to increase the degradation. In any event, a chlorophyll sample must be studied spectroscopically before confidence can be placed in analytical values obtained by its use as a calibration standard. There are also problems involved in the satisfactory storage of the pigment.

A calibration curve obtained from a typical plant extract as described above will be valid for most of the usual plants. Where there is an abnormal chlorophyll *a* to *b* ratio the calibration should be made using an extract of this substance or of one that approximates it in the *a* to *b* ratio.

Although a specific type of photoelectric colorimeter was employed in this work, there is no reason why similar results should not be obtainable with other instruments of equivalent precision employing appropriate filters. If a suitable spectrophotometer is unavailable this method of calibrating a photoelectric colorimeter can still be used conveniently, provided it can be arranged for another laboratory to make the measurements at 6600 and 6425 Å. required to evaluate the chlorophyll concentration. The worker desiring this information can prepare the ether solutions of appropriate concentrations and ship them in tightly corked bottles packed in dry ice by air express to the laboratory making measurements. Zscheile, Comar, and Mackinney (*8*) exchanged samples satisfactorily in this manner between Indiana and California, and it has been found that such ether solutions can be stored for at least 3 months without change of analytical results (*2*). The colorimetric readings on the acetone solutions should be made immediately after preparation of the plant extract and the calculations can be made at a later date after receipt of the spectrophotometric data.

Since the colorimetric results are dependent to such a large degree on the spectrum of the chlorophyll standard, it is evident that satisfactory interlaboratory agreement will not be attained until all laboratories employ standards with identical absorption properties for the optical systems used. In view of the characteristics of this pigment, calibration as herein described by means of the plant extract and the spectrophotometric method seems to offer an immediate practical solution to the problem.

These principles apply to colorimetric analysis in general and this procedure may be advantageously employed in other methods where the calibration standards are of unstable character or difficult to obtain. Work designed to demonstrate the broad application of these principles is under way.

Summary

A comparison is presented of the absorption spectra of three chlorophyll preparations and the analytical results obtained when these preparations were used as standards for the calibration of a photoelectric colorimeter. Analytical values from 24 different plant sources show that deviations as high as 75 per cent from the true values may occur, depending on the purity of the standard.

A simple plant extract may be used for calibration purposes where it is possible to have an aliquot of the extract analyzed for chlorophyll, using an established spectrophotometric method and published absorption coefficients, thus avoiding the uncertainties involved in the use of chlorophyll preparations as standards.

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Quantitative Chemical Microdetermination of Twelve Elements in Plant Tissue

A Systematic Procedure

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A systematic procedure of analysis is presented for the quantitative chemical microdetermination of twelve nutritionally important elements on single samples of plant tissue. The elements determined are calcium, magnesium, potassium, sodium, phosphorus, sulfur, iron, zinc, copper, manganese, molybdenum, and cobalt. Data are presented supporting the validity of the separatory procedures and analytical methods used, and their arrangement into a scheme of systematic analysis.

INVESTIGATIONS being carried out at this laboratory on the relationships of soil fertility to the production of food crops of high nutritive value are concerned with both the macro- and the micronutrient elements. A fairly complete study of these interrelationships necessitates an extensive analytical program. It would be desirable to have analyses for all the mineral elements commonly accepted as being essential for the growth of either plants or animals. The procedure presented here is for the analysis of calcium, magnesium, potassium, sodium, phosphorus, sulfur, iron, manganese, zinc, molybdenum, copper, and cobalt. It is hoped that future work will make possible the inclusion of other nutritionally important elements in some such system of analysis.

An examination of published analytical procedures reveals a number of excellent methods for the individual determination of these elements. The adoption of these methods in the routine determination of such a list of elements on each of hundreds of samples of plant material is impracticable. Many of the proposed analytical methods are designed for the determination of but one or two elements on the ash of a sample of plant material; hence the determination of a dozen elements would require at least two or three separate ashings of each sample. This involves not only considerable time and labor, but also an amount of sample which is often not available. This is particularly true in certain greenhouse pot studies, where an extensive mineral analysis is desired on a sample consisting of only a few plants or plant parts. This situation has been confronted in the majority of the soil and sand pot studies at this laboratory, in which it was desired to make determinations on the individual replicates of a given treatment.

The scheme of analysis reported in this paper was developed to make possible quantitative analyses for twelve nutritionally important elements on single samples of only 5 to 10 grams of dried plant material. The procedure developed is shown schematically in Figure 1. In place of several dry ashings at different temperatures, and with additions of various chemicals (as recommended in many individual analytical methods), a single 5- to 10-gram sample of dried plant material is digested with nitric and perchloric acids, and then treated with hydrofluoric acid to remove silica. The residue is dried and dissolved in dilute hydrochloric acid. The resultant solution (solution A) contains all the elements to be subsequently determined.

Phosphorus, iron, manganese, and molybdenum are run directly on solution A. The remainder of this solution is made alkaline and extracted with dithizone in carbon tetrachloride. The aqueous phase (solution C) contains the alkali metals, alkaline earths, and sulfur. The carbon tetrachloride extract (solution B) contains cobalt, copper, and zinc. Zinc is separated from cobalt and copper by an acid dithizone extraction of solution B. Copper and cobalt are determined on the carbon tetrachloride phase of this extraction. Sulfur is determined gravimetrically on an aliquot of solution C. A second aliquot of solution C is digested with acids to remove ammonia and citrates, giving solution F. Sodium and potassium determinations are made on two separate portions of this solution. A third aliquot of solution F is treated with ferric chloride and ammonium hydroxide to remove aluminum, phosphorus, and iron. Calcium is determined on the resultant solution, and magnesium is run on the solution remaining after the calcium precipitation.

In the selection of the individual procedures used, a number of published methods for each element were critically examined under the conditions of the proposed systematic procedure. Colorimetric methods were favored because of their sensitivity and speed. The final selections of methods were made for accuracy in the range desired, size of sample required, speed, and adaptability to systematic analysis. This last requirement is a particularly difficult and exacting one, since each individual determination must be so constituted or so placed in the scheme that elements to be determined subsequently are neither added nor removed. Also, reagents added in the determination must introduce no significant impurities or compounds which interfere with subsequent determinations. All the methods used are adaptations or modifications of published methods. Most of the changes were designed to make the methods applicable to different apparatus or to concentration ranges other than those for which they were originally proposed. The analytical scheme and methods used are described in the following section without comment, a discussion of the developments and details of the scheme is given later, and a separate section is devoted to special precautions and techniques.

Analytical Procedure

The purification of all solutions, reagents, and apparatus used in element microdeterminations and in all steps preceding them requires particular care (see section on Special Precautions and

Table I. Pertinent Data on Methods Used

Element	Units	Range of Method[a]	Tissue in Aliquot	Volume of Color Solutions	Cell Dimensions Minimum volume	Cell Dimensions Depth	Wave Length for Color Comparisons[b]	Methods Used
			Grams	*Ml.*	*Ml.*	*Cm.*	*Mμ*	
Ca	Mg.	0.02–2.0	0.042	...	..	...	Volumetric	Permanganate
Mg	γ	1–50	0.005	50	10	1.7	650	Quinolate
K	Mg.	2–8	0.270	100	10	1.7	400	Cobaltinitrite-dichromate
Na	Mg.	0.01–0.36	0.054	50	10	1.7	440	Zinc uranyl acetate
P	Mg.	0.001–0.06	0.006	10	10	1.7	600	Molybdate
S	Mg.	2–100	2.700	...	..	...	Gravimetric	Barium chloride
Fe	γ	1–60	0.060	10	10	1.7	490	*o*-Phenanthroline
Zn	γ	0.2–20	0.162	10	10	1.7	540	Dithizone
Cu	γ	1–60	1.080	10	10	1.7	430	Diethyldithiocarbamate
Mn	γ	5–200	0.600	11	10	1.7	525	Periodate
Co	γ	0.2–10	4.320	5	3	5.0	500	Nitroso R salt
Mo	γ	0.3–15	1.200	25	25	5.0	475	Thiocyanate

[a] As used in this schematic procedure.
[b] Determined by spectral transmission curves.

Techniques). A Coleman Model 11 Universal spectrophotometer with several types of cuvettes is used for all colorimetric measurements. The appropriate wave lengths for all color observations were obtained by preparing spectral transmission curves. Table I contains data on wave band, cell dimensions, and range for all methods used. The procedure is described in terms of a single determination for each element on one 7.5-gram sample.

Preparation of "Ash" Solution. Weigh a 7.50-gram sample of plant tissue into a 600-ml. Pyrex beaker. (Pyrex ware is used throughout the procedure, except for the storage of dry samples, and of concentrated acids.) Add 12.5 ml. of concentrated nitric acid, cover with a watch glass, and evaporate to near dryness on a steam plate. One repetition of this digestion is usually enough. Add 25 ml. of 1 to 1 nitric acid and 25 ml. of 60 per cent perchloric acid and boil (covered with watch glass) until nearly dry. Add about 20 ml. of water and transfer quantitatively to a 125-ml. platinum dish. Add 5 ml. of perchloric acid to the beaker, boil, and add to the solution in the platinum dish. Add 2 to 8 ml. of 48 per cent hydrofluoric acid, using a paraffin-coated dipper. Place the dish in a sand bath on a gas hot plate and heat carefully until fumes are no longer given off. Cool and transfer to a 400-ml. beaker, using small additions of hot, 0.6 *N* hydrochloric acid. Add more hot hydrochloric acid to give about 100 ml. and dissolve by continued heating and crushing of solid material with a flat-ended glass rod. This operation is essential to assure complete solution of calcium sulfate and other salts. If insoluble salts persist, increase the concentration of hydrochloric acid. Dilute to a 125-ml. volume and label solution A.

Determinations on Solution A. *Molybdenum* is determined by an adaptation of the methods of Marmoy (*19*) and Rogers (*25*).

Pipet a 20.0-ml. aliquot of solution A into a 200-ml. separatory funnel. Add 30 ml. of 1 to 7 hydrochloric acid, 3.0 ml. of aqueous 10 per cent potassium thiocyanate, and 3.0 ml. of fresh 10 per cent stannous chloride in 1 to 9 hydrochloric acid. Shake after each addition. After 2 minutes add 20 ml. of diethyl ether. (The ether for a set of determinations is freshly extracted with about one tenth its volume of 1 to 1 stannous chloride–potassium thiocyanate mixture and washed until clear with 1 to 9 hydrochloric acid.) Shake vigorously for 30 seconds, and drain the aqueous layer into a similar funnel. Shake again with 15 ml. of extracted ether. Rinse the ether extracts into a 25-ml. volumetric flask and dilute to volume with extracted ether. Sufficient evaporation will have taken place to make this possible. Compare colorimetrically at once with at least two standards prepared simultaneously to establish the slope of the curve. Read against extracted ether as a reference liquid.

Manganese is determined by the simplified periodate method described by Peech (*22*).

Pipet 10.0 ml. of solution A into a 50-ml. beaker, and evaporate to dryness on a steam plate to remove hydrochloric acid. Dissolve in 6 ml. of 1.0 *N* nitric acid and transfer to a test tube graduated at 11.0 ml. Add 1 ml. of 85 per cent orthophosphoric acid and dilute to volume. Place the tube in a boiling water bath, add about 50 mg. of solid sodium periodate, mix with a glass rod, and heat in the bath for 1 hour. Cool, make to volume, mix, and compare color intensities against a reagent blank reference liquid. The working curve is constant, but may be checked by the inclusion of one or two standards with each set of samples.

Iron is determined by the method of Saywell and Cunningham (*28*).

Pipet a 1.00-ml. aliquot of solution A into a test tube graduated at 10.0 ml. Add 1 ml. of 10 per cent hydroxylamine hydrochloride solution and 0.5 ml. of *o*-phenanthroline (1.5 per cent in 95 per cent ethanol). Add a small square of Congo red indicator paper, and adjust to the alkaline end point with 1 to 5 ammonium hydroxide. Avoid the formation of a precipitate which will result from the addition of excess hydroxide. Make to volume, mix, and read the per cent transmittance, using a reagent blank as a reference liquid. The working curve remains constant.

Phosphorus is determined by an adaptation of the method of Fiske and Subbarow (*12*) and McCune and Weech (*18*).

Pipet a 0.100-ml. aliquot of solution A into a test tube graduated at 10.0 ml., using a 0.100-ml. Mohr pipet. Add 1 ml. of water to the test tube, and discharge the pipet by blowing, with the tip under water. Add 1.0 ml. of 2.5 per cent ammonium molybdate in 5 *N* sulfuric acid. Mix and add 0.4 ml. of 0.25 per cent 1,2,4-aminonaphtholsulfonic acid (prepared by adding 0.125 gram of animonaphtholsulfonic acid to 49 ml. of filtered 15 per cent sodium bisulfite, and then adding 1.25 ml. of 20 per cent sodium sulfite, *12*). Make to volume and mix. Prepare a set of standards simultaneously, since the slope of the standard curve is not constant. Allow color to develop 30 minutes before reading. Use distilled water as a reference liquid.

Determinations on the Dithizone Solutions. *Dithizone separations.* The dithizone (diphenylthiocarbazone) is purified and the separations are made by a method similar to those described by Bambach and Burkey (*3*), Cowling and Miller (*7*), Holland and Ritchie (*14, 15*), and Sandell (*26, 27*).

The dithizone reagent used has a concentration of 0.5 gram per liter, which is several times more concentrated than is ordinarily used. To prepare this stock reagent, dissolve 0.5 gram of commercial dithizone in about 500 ml. of carbon tetrachloride. Filter into a 5-liter separatory funnel containing 2 to 3 liters of 0.02 *N* ammonium hydroxide. Shake well, extracting the dithizone into the aqueous phase. Separate the carbon tetrachloride, and use to dissolve the dithizone remaining on the filter paper. Filter again into the same separatory funnel. Repeat this process until little residue remains on the filter paper. After the last extraction, discard the carbon tetrachloride phase. Extract the ammoniacal solution of dithizone with fresh 50-ml. portions of carbon tetrachloride until traces of pink no longer appear in the carbon tetrachloride layer. Add about 500 ml. of carbon tetrachloride and acidify with hydrochloric acid, with shaking, until the dithizone is extracted from the aqueous phase into the carbon tetrachloride phase. (If necessary, add more acid and carbon tetrachloride and repeat.) Dilute to 1 liter with carbon tetrachloride. Store in a glass-stoppered bottle, in a cold, dark place (*5*). This reagent should remain stable for several weeks.

Place a 90.0-ml. aliquot of solution A in a 500-ml. separatory funnel. Dilute with 50 ml. of redistilled water. Add enough 40 per cent ammonium citrate solution (prepared using lead-free citric acid plus concentrated ammonium hydroxide to pH 8.5, and extracted with dithizone) to prevent precipitation when the solution is subsequently made alkaline. Usually 10 ml. are sufficient. Adjust to pH 8.5 with about 7 *N* ammonium hydroxide, using a few drops of *m*-cresol purple (1 mg. per ml. in ethanol) as an internal indicator. It may be necessary to add 15 or 20 drops of indicator, if the solution turned yellow on the addition of citrate. Add an excess of concentrated dithizone reagent (usually 20 to 25 ml.) as indicated by a strongly orange aqueous phase after shaking. Drain the carbon tetrachloride phase into a similar, dry separatory funnel, rinsing the last portion through with carbon tetrachloride. Extract the aqueous phase with successive 25-ml. portions of carbon tetrachloride (maintaining an excess of dithizone in the aqueous layer), until the carbon tetrachloride layer is clear green, and combine all the extracts. This is solution B.

Wash the aqueous phase into a 200-ml. volumetric flask with 10 ml. of 1 to 1 hydrochloric acid, dilute to 200 ml., and save for subsequent determinations of sulfur, calcium, magnesium, potassium, and sodium. Designate as solution C.

Into the combined carbon tetrachloride extracts (solution B) in a 500-ml. separatory funnel, pipet 50.0 ml. of 0.02 *N* hydro-

chloric acid. Shake vigorously for 2 minutes (within 5 or 10 seconds). Separate the carbon tetrachloride phase into a 250-ml. beaker, and rinse the funnel with carbon tetrachloride. Drain the aqueous phase into a dry container and save for the subsequent determination of zinc (solution E). [This solution may also be used for the determinations of cadmium (*27*) and lead (*26*).]

Add 5 ml. of 60 per cent perchloric acid to the carbon tetrachloride phase and boil over a low flame until fumes of perchloric acid appear. Cover with a watch glass and digest until clear. Remove the watch glass and continue boiling until dry. Cool and dissolve in 12.5 ml. of 0.04 molar citric acid (C. P. lead-free). (Most samples of C. P. citric acid contain appreciable amounts of copper and other metals, and must be purified.) Dilute to 25.0 ml. and use for the determinations of cobalt and copper (solution D).

Copper is determined by a modification of the procedures of Coulson (*6*), Drabkin (*10*), and Marston and Dewey (*20*).

Transfer the remaining cobalt and copper solution (D) into a 60-ml. separatory funnel. Add 2 ml. of 40 per cent ammonium citrate adjusted to pH 8.5, 10 ml. of 0.1 per cent sodium diethyldithiocarbamate, and 10.0 ml. of isoamyl acetate. [Eden and Green (*11*) reported the necessary presence of ammonium ions to deionize iron. The presence of ammonium ions also seems to be necessary to prevent rapid fading of the copper color complex.] Shake for 5 minutes, separate the acetate layer, and centrifuge it for 5 minutes to remove water droplets. (All centrifuging is done at 2000 r. p. m., using an International Equipment Company laboratory centrifuge, Size 1, Type SB. The head is 13 cm. from axis to trunnion carrier pivot.) Read against a reference liquid of isoamyl acetate. A few standards should be included with each run to check the working curve.

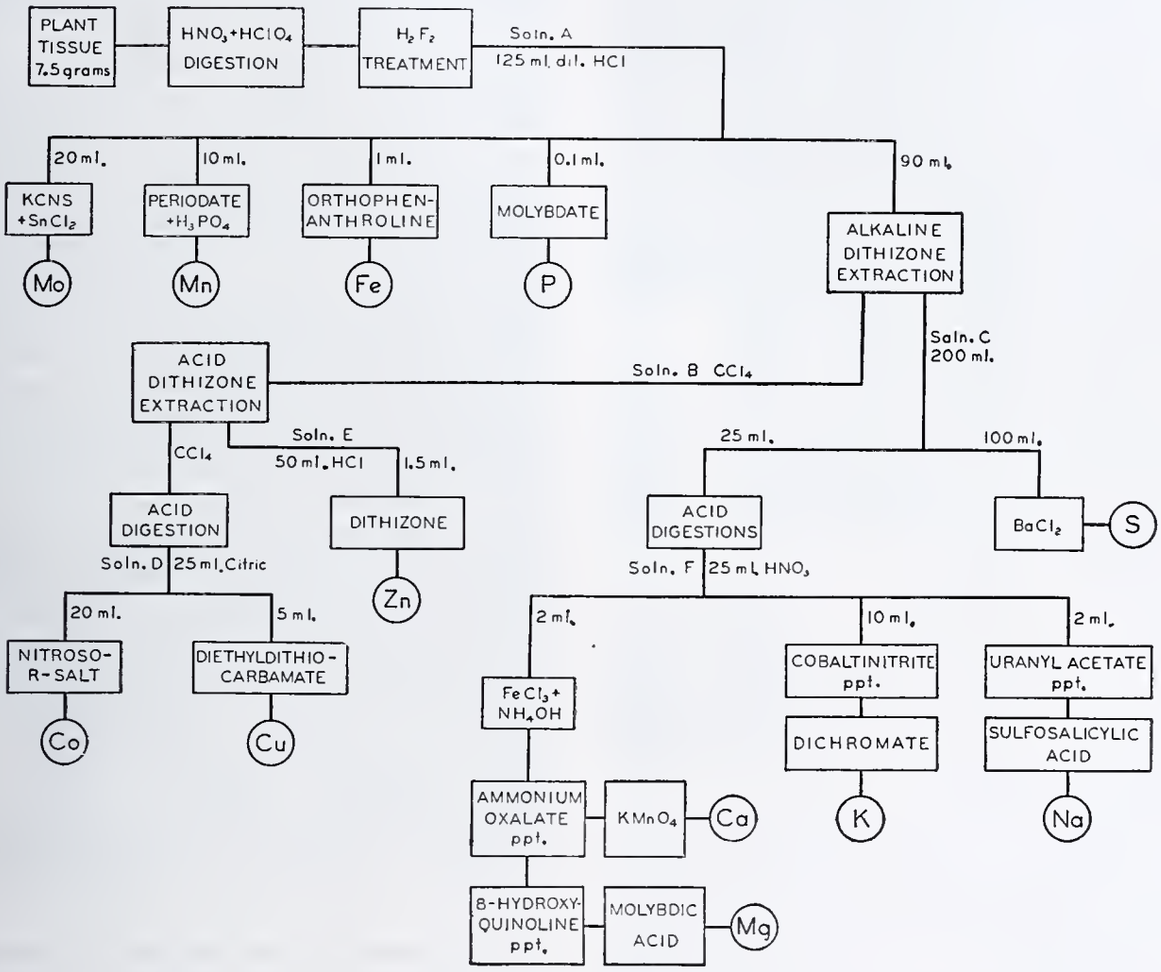

FIGURE 1. SCHEMATIC DIAGRAM OF PROCEDURE

Cobalt is determined by a modification of the method described by Marston and Dewey (*20*).

Pipet 20.0 ml. of the cobalt and copper solution (D) into a 50-ml. Pyrex beaker and rinse the pipet back into the remaining solution. Evaporate to about 5 ml. and add 2.5 ml. of buffered soda solution (6.184 grams of boric acid, 35.62 grams of disodium phosphate dihydrate, and 500 ml. of 1 *N* sodium hydroxide, diluted to 1 liter). The pH should be between 8.0 and 8.5 and may be checked externally with phenol red and *m*-cresol purple. Add 1 ml. of 0.2 per cent nitroso R salt slowly with mixing. Heat to boiling for 1 to 2 minutes, add 2 ml. of 1 to 1 nitric acid, and boil for another 1 to 2 minutes. Add 0.5 to 1 ml. of saturated bromine water and let stand warm for 5 minutes. Cover with a watch glass, and boil for 2 or 3 minutes to remove the excess bromine. Cool, transfer, and dilute to 5.0 ml. Intense colors may be further diluted before reading. Where the cobalt is very low, it may be possible to keep the volume to 4 or even 3 ml. Read color intensity within a few hours against a distilled water reference. A few standards should be included in each run to check the working curve.

Zinc is determined by an adaptation of the method of Cowling and Miller (*7*).

Pipet a 1.5-ml. aliquot of solution E into a 60-ml. separatory funnel. Add 23.5 ml. of 0.02 *N* hydrochloric acid. Add by pipet, 25.0 ml. of buffer solution [9 parts of (I), a stable solution containing 500 ml. of 0.5 molar, dithizone-extracted ammonium citrate plus 150 ml. of 1 *N* ammonium hydroxide, diluted to 2250 ml.; and 1 part of (II), freshly prepared 0.25 per cent sodium diethyldithiocarbamate]. Add exactly 5.0 ml. of dithizone reagent (concentrated reagent diluted 1 to 4 with carbon tetrachloride). Shake for 5 minutes. Collect the carbon tetrachloride layer in a 10-ml. beaker, dilute a 2.0-ml aliquot to 10.0 ml. with carbon tetrachloride, and read per cent transmittance against a carbon tetrachloride reference. This zinc procedure should be carried out in a darkened room and the colors read within an hour. A standard curve should be prepared with each set of samples.

SULFUR, ALKALIES, AND ALKALINE EARTHS. *Sulfur* (*2*). Place a 100.0-ml. aliquot of solution C in a 400-ml. beaker. Add about 100 ml. of water and a few drops of bromocresol green,

neutralize with concentrated ammonium hydroxide. Then add 0.5 ml. of concentrated hydrochoric acid. Bring to a boil, add 10 ml. of 10 per cent barium chloride dropwise with constant stirring, and boil for another 5 minutes. Cover with a watch glass, and let stand for at least 5 hours in a warm place (steam plate). Transfer the precipitate to an asbestos pad in a weighed Gooch crucible and wash with boiling water until free of chlorides. Ignite at 600° C., and weigh as barium sulfate.

Removal of Ammonia, Iron, Aluminum, and Phosphorus. These steps are modified from the work of Peech (*22*).

Evaporate 25.0 ml. of solution C to dryness on a steam plate, in a 150-ml. beaker. Digest with 10 ml. of freshly prepared aqua regia (1 part of concentrated nitric acid and 3 parts of concentrated hydrochloric acid) and heat to dryness. Digest with perchloric acid (5 ml. are usually sufficient), and boil to dryness. Take to dryness twice more with 5-ml. portions of aqua regia to remove any residual ammonia. Take up and dilute to a 25-ml. volume with 0.1 *N* nitric acid. This is solution F.

For the determinations of calcium and magnesium transfer a 2.0-ml. aliquot of this solution to a 15-ml. centrifuge tube graduated at 13.0 ml., and save the remainder for the determinations of sodium and potassium. Add 0.2 ml. of ferric chloride solution (1.22 grams of ferric chloride hexahydrate in 250 ml. of 1 to 250 hydrochloric acid), mix, add 8 ml. of buffer solution (25 grams of sodium acetate, 62.5 grams of ammonium chloride, and 0.5 gram of sodium hydroxide in 1 liter of solution), and mix again. Add 1 drop of methyl red indicator solution (0.02 per cent) and 0.6 *N* ammonium hydroxide until the color of the solution changes from slightly red to deep yellow, and then add 2 drops in excess. Dilute to about 13.2 ml., mix with a stirring rod, and digest in a water bath at 80° C. for 5 minutes to flocculate the precipitate. Mix thoroughly, and centrifuge while hot for 10 minutes. The solution should have evaporated to 13.0 ml.

Calcium is determined by the method of Peech (*22*).

Transfer a 10.0-ml. aliquot of the clear supernatant solution (using inclined mirror to observe liquid level while pipetting, *22*) into another calibrated centrifuge tube. Add 1.4 ml. of 0.2 *N* hydrochloric acid and place in a water bath at 70° C. Mix the contents, add 2 ml. of 3 per cent ammonium oxalate, mix thoroughly again, and digest for 30 minutes at 70° C. Remove the tube from the bath and let stand for 30 minutes. Centrifuge for 15 minutes. The volume should now be 13.0 ml. Decant the clear supernatant liquid gently into a dry test tube and save for the magnesium determination.

Allow the centrifuge tube to drain for several minutes on paper toweling. Add from a wash bottle, so as to break up the precipitate, about 5 ml. of 2 *N* ammonium hydroxide saturated with calcium oxalate. Centrifuge for 15 minutes, decant carefully and discard the solution. Drain the tube and save the precipitate. One washing is sufficient unless very large quantities of calcium are present. Add about 5 ml. of 10 per cent sulfuric acid, heat to 70° C. in a water bath, and titrate with standard 0.02 *N* potassium permanganate.

Magnesium is determined by the method of Peech (*22*).

Pipet a 10.0-ml. aliquot from the solution set aside for the magnesium determination into a 15-ml. centrifuge tube graduated at 13.0 ml. Place the tube in a water bath at 70° ± 2° C., add 0.8 ml. of a freshly prepared 2 per cent alcoholic solution of 8-hydroxyquinoline. Mix immediately and then add 0.4 ml. of concentrated ammonium hydroxide. Stir vigorously (1 minute or longer) until full turbidity develops. Set aside until all samples of the set are precipitated. Replace the centrifuge tube in a water bath at 70° C. for 10 minutes to flocculate the precipitate. After digestion, cool by immersing the centrifuge tube in a bath at about 25° C., and allow to stand for 45 minutes to assure complete precipitation of magnesium. Add 0.5 ml. of 95 per cent ethyl alcohol slowly down the sides of the centrifuge tube, rotating the tube at the same time to wash down the precipitate and to form a layer of alcohol on the surface of the solution. Centrifuge for 15 minutes and by using gentle suction draw off twice 2 to 3 ml. of the clear liquid to remove the layer of alcohol.

Decant carefully and discard the solution, wipe the mouth of the tube with filter paper, add 5 ml. of ammoniacal ammonium acetate (8 ml. of concentrated ammonium hydroxide in 300 ml. of 0.7 *N* ammonium acetate), wash the solution down the sides of the tube and break up the precipitate. Add 0.5 ml. of ethyl alcohol to prevent creeping and centrifuge again. Repeat the washing once more. Dissolve the precipitate in 4 ml. of 0.5 *N* hydrochloric acid, dilute to 13.0 ml. with water, and mix. Place a 2.00-ml. aliquot of this solution in a 50-ml. volumetric flask. Dilute to about 35 ml. with water and add 5 ml. of 20 per cent sodium carbonate solution and 3 ml. of phenol reagent (750 ml. of water, 100 grams of sodium tungstate, 20 grams of phosphomolybdic acid, and 50 ml. of 85 per cent phosphoric acid, boiled for 2 hours, and diluted to 1 liter). Mix the contents after each addition. Place the flask in boiling water for 1 minute, and cool for 15 minutes. Dilute to volume, mix, and read, using a reagent blank as reference. The working curve may be checked by including a few standards with each run.

Potassium is determined by the method of Wander (*32*).

Place a 10.0-ml. aliquot of solution F in a 25 × 150 mm. centrifuge tube, add 5 ml. of 20 per cent trisodium cobaltinitrite (freshly prepared and filtered), mix, and keep at 20° C. for exactly 2 hours. Wash down the walls of the tube with 0.01 *N* nitric acid. Centrifuge for 10 minutes (0.01 *N* nitric acid is used in balancing the tubes before centrifuging), decant, and allow the tube to drain for several minutes. Wash the precipitate thoroughly with 15 ml. of 0.01 *N* nitric acid, centrifuge, and drain as before. Add 10.0 ml. of standardized 0.1 *N* potassium dichromate and 5 ml. of concentrated sulfuric acid. Mix thoroughly, cool to room temperature, and make to 100-ml. volume. Mix and read the color against an 11-mg. potassium standard as reference, since this gives a completely reduced dichromate solution. The color measured is that of the unreduced dichromate. Several potassium standards are carried throughout the procedure.

Sodium is determined by the method of Darnell and Walker (*8*) and Broadfoot and Browning (*4*).

Place a 2.00-ml. aliquot of solution F (for samples with very high sodium content, use 1.0 or 0.5 ml.) in a 15-ml. centrifuge tube. Add 5 ml. of freshly filtered uranyl zinc acetate reagent. [Prepare by mixing while warm equal parts of (I), a solution of 77 grams of uranyl acetate plus 14 ml. of glacial acetic acid in 400 ml., with (II), a solution of 231 grams of zinc acetate plus 7 ml. of glacial acetic acid in 400 ml. Let stand at least 24 hours and filter.] At 5-minute intervals, add seven 0.3-ml. portions of ethyl alcohol. These additions must occupy at least 30 minutes. After each of the first five additions of alcohol, mix the liquid in the tube thoroughly. The last two additions of alcohol serve to wash down the walls of the tube and are allowed to remain layered on the solution. After the last addition of alcohol, centrifuge the tube for 10 minutes, decant, invert, and allow to drain for 5 minutes. Wipe the mouth of the tube dry and agitate the precipitate by directing on it a fine stream of about 2 ml. of ethyl acetate–acetic acid wash liquid (300 ml. of ethyl acetate diluted to 1 liter with glacial acetic acid). Wash down the walls of the tube with about 5 ml. of the wash liquid, centrifuge, drain, and wipe as before. Wash the precipitate and the walls of the tube with about 5 ml. of diethyl ether. Centrifuge for 5 minutes, decant, and drain for not more than 1 minute (prolonged drying will result in the dropping of the precipitate from the tube). Wash the precipitate a second time with 5 ml. of ether, centrifuge 5 minutes, decant, and drain as before. Put the tube in a warm place for 5 minutes to evaporate the last traces of ether.

Dissolve and transfer with water the washed, dried precipitate to a 50-ml. volumetric flask. Dilute the solution to about 35 ml. and to it add, in order, 2 ml. of 5 per cent sulfosalicylic acid, 2 ml. of 10 per cent sodium acetate trihydrate solution, and water to make 50 ml. Mix the solution and read, using as a reference a solution containing 2 ml. of 5 per cent sulfosalicylic acid and 2 ml. of 10 per cent sodium acetate diluted to 50 ml. with water. The working curve should be checked by including three or four sodium standards with each set of determinations.

Special Precautions and Techniques

It is not possible to overemphasize the care which must be exercised in the purification of all apparatus, solutions, and reagents used in all the steps preceding the element microdeterminations, and in the determinations themselves. Pyrex glassware is used exclusively, and redistilled water is used throughout the procedure for all solutions and for rinsing

Table II. Recovery Determinations on Turnip Greens

Element	Units	Present	Added	Total Present	Total Found	% Difference
Ca	Mg./g.	40.0	10.0	50.0	51.2	+ 2.4
Mg	Mg./g.	2.54	2.00	4.54	4.62	+ 1.8
K	Mg./g.	24.6	10.0	34.6	33.3	− 3.7
Na	Mg./g.	5.27	2.00	7.27	7.39	+ 1.6
Pe	Mg./g.	4.65	2.00	6.65	6.71	+ 0.9
S	Mg./g.	12.1	2.0	14.1	13.7	− 2.8
F	γ/g.	147	40.0	187	193	+ 3.2
Zn	γ/g.	42.0	11.1	53.1	51.5	− 2.9
Cu	γ/g.	7.2	2.7	9.9	10.2	+ 3.0
Mn	γ/g.	35.4	16.7	52.1	54.9	+ 5.4
Co	γ/g.	0.07	0.08	0.15	0.18	+16.6
Mo	γ/g.	4.94	5.00	9.94	9.80	− 1.4

all glassware. This water is prepared by use of a continuously operating all-glass still similar to those described by Piper and Oertel (*23*). This water is stored in and dispensed from an all-glass suction-operated siphon apparatus, in which the siphon dispenser and the vacuum line both end in a plastic-coated two-hole stopper. The vacuum system is closed by placing the receiving flask against this stopper. In this manner the redistilled water may be stored and dispensed without contact with rubber or cork.

All ammonium hydroxide, hydrochloric acid, and nitric acid (except concentrated acids), and all organic solvents not purified by extraction (such as ether in the molybdenum determination) are purified by distillation from glass. All carbon tetrachloride should be distilled over calcium oxide to remove acidifying substances. All solutions used in heavy metal determinations come in contact only with glassware which has been rigorously cleaned with aqua regia or hot nitric acid. All ground-glass joints require particular care to accomplish this cleaning. Glass stopcocks usually require a further extraction with dithizone. It is also imperative that none of the solutions or apparatus come in contact with rubber at any point during the procedure. Rubber contacts are eliminated from all wash bottles, and only glass-stoppered volumetric flasks, separatory funnels, and reagent bottles are used.

A digestion and reagent blank must be carried throughout the entire scheme with each set of samples. Not only does the trace metal content of different lots of chemicals vary markedly, but the content of impurities of the reagent solutions may also change with storage in glass apparatus. Extractions are facilitated by the use of two-shelf separatory funnel racks, in which the upper shelf is adjustable in height and may be removed for separate use. The funnels can be clamped into the racks for shaking, with sponge-rubber padded cross-bars. A standard reciprocal shaking machine has been modified to hold two shelves of these racks of separatory funnels in a horizontal position. Thus, the dithizone, zinc, and copper extractions, which are normally rather time-consuming, can be carried out 24 at a time with uniform and complete extraction.

The centrifuge tube techniques and water bath designed by Peech (*22*) for microanalytical procedures are used to excellent advantage. The conical 15 × 125 mm. centrifuge tubes are graduated at 13.0 ml. by circling with a diamond point. Similarly graduated test tubes are extensively used in place of (and are more adaptable than) small volumetric flasks. The two sizes most commonly used are 15 × 125 mm., graduated at 10.0 and 11.0 ml., and commercially available 10 × 115 mm. tubes, graduated at each milliliter from 1 to 8.

Results

The characteristics and applicability of the proposed scheme of analysis are shown by the data in the following tables. The recoveries of elements added to plant tissue are given in Table II. Thirteen portions of a well-mixed sample of turnip greens were used in this comparison. An increment of but one of the twelve elements to be determined was added to each of twelve samples, while the last sample had no additions. All samples were then run completely through the schematic procedure described. The values reported are the average of duplicate analyses. The agreement between the "Total Present" and "Total Found" for the different elements can be considered as very satisfactory for most types of plant tissue analysis. The difference between these two values for any determination was less than 6 per cent, except for cobalt. The large percentage difference for cobalt is but a small actual difference and represents approximately the reliability (0.02 microgram per gram) of the method used. These recovery determinations not only indicate the precision of the individual methods, but in addition measure losses or contaminations in the sample digestion and in all separations and manipulations, as well as in the actual determinations of the element.

The proposed system of analysis was further checked by comparison with referee samples. The data in Table III were obtained by analysis of six referee samples of different types of plant material, all carefully prepared and analyzed in other laboratories. These analyses were obtained by ashing techniques and chemical methods different from those used in this study. These samples were likewise carried through all operations of the analytical scheme, and the average of duplicate determinations was reported. The analyses are in satisfactory agreement with the results of other investigators, since for all elements but cobalt the differences are less than 8.5 per cent.

Table III. Determinations on Referee Samples

Element	Units	Authors' Analyses	Analyses and Types of Methods of Other Laboratories	% Difference	Type of Material
Ca	Mg./g.	2.97	2.99 (*22*)	0.7	Cauliflower
Mg	Mg./g.	3.47	3.36 (*24*)	3.2	Alfalfa
K	Mg./g.	15.00	13.86 (*24*)	7.6	Alfalfa
Na	Mg./g.	3.76	3.67 (*8*)	2.5	Sugar beet top
P	Mg./g.	2.03	2.09 (*31*)	3.0	Alfalfa
S	Mg./g.	4.18	3.85 (*2*)	7.8	Tomato leaflet
Fe	γ/g.	81.2	78.4 (*2*)	3.5	Turnip top
Zn	γ/g.	25.8	23.8 (*7*)	8.4	Alfalfa
Cu	γ/g.	11.4	11.8 (*6*)	3.4	Alfalfa
Mn	γ/g.	32.0	34.7 (*24*)	8.4	Alfalfa
Co	γ/g.	0.07	0.08 (*16*)	12.5	Turnip top
Mo	γ/g.	No sample available			

Discussion

The analytical methods incorporated into the proposed scheme of analysis have been drawn mainly from published literature pertaining to soil and plant tissue analysis. Some modifications were necessary to make the methods applicable to smaller quantities of material than originally proposed. Other changes were made to adapt the methods to rapid schematic analysis. At the same time it was possible to eliminate some of the preliminary operations necessary for the removal of interfering substances. Some of the colorimetric methods were originally developed for filter colorimeters or visual comparison. In some cases the sensitivity of these methods has been improved by the use of a spectrophotometer, greater cell depth, and smaller final volumes.

The use of dry-ashing techniques in the determination of such a list of elements would necessitate at least two samples, since the magnesium or calcium salt which must be added to prevent losses of sulfur and phosphorus would interfere with subsequent analyses for these elements, and for the trace metals. This type of replicate ashing consumes extra time, apparatus, and sample. The wet-digestion procedure used (modified from Gieseking, Snider, and Getz, *13*) has proved to be both rapid and smooth in operation. The resultant solution from such a single digestion contains, without losses, the twelve elements under consideration. The use of nitric acid in this digestion precludes the determination of nitrogen. The removal of silica has generally been accepted as necessary (*9*, *21*), and is accomplished by volatilization with hydrofluoric acid. If desired, a measure of crude silica may be obtained by measuring the loss in sample weight due to the hydrofluoric acid treatment. Since boron is also volatilized as the fluoride, boron as well as nitrogen must be determined on a separate sample when such an analysis is desired.

The use of perchloric instead of sulfuric acid in the removal of residual fluorides forms more readily soluble salts, and makes possible the subsequent determination of sulfur.

An ether extraction of molybdenum and iron is not used on the entire "ash" solution, since the partial extraction of phosphorus which occurs (*17*) would make its subsequent analysis impossible. The ether–stannous chloride–potassium thiocyanate extraction used for molybdenum is not made on the solution in which other elements are to be determined, because of the presence of relatively large amounts of trace metal impurities in the stannous chloride. Attempts to determine molybdenum on solution C have not been completely successful. The results shown in the following table tend to indicate that this method can be made to work satisfactorily:

Sample No.	Molybdenum Content: Solution A, by standard method	Molybdenum Content: Solution C, HNO_3 and $HClO_4$ treatment, determined by H_2SO_4 method
	γ/g.	γ/g.
164	4.4	4.5
165	4.4	4.2
184	0.9	1.3

The molybdenum thiocyanate color complex is subject to fading due to air oxidation. This effect may be minimized by the use of glass-stoppered volumetric flasks, and by comparison within a few minutes after extraction. Faded colors may be fairly accurately restored by shaking with stannous chloride–potassium thiocyanate solution.

Unsuccessful attempts were made to determine manganese on the aqueous phase of the molybdenum extraction. It was not possible to effect the oxidation of tin salts and removal of chlorides (to prevent reduction of sodium periodate added in the manganese determination) without occlusion of part of the manganese in the precipitate formed. Attempts to determine manganese on solution C were more promising, but not conclusive. It is felt that it may subsequently be found possible to determine manganese on solution C.

Although iron and phosphorus may be determined on the aqueous phase of the alkaline dithizone extraction, the very small amount of sample required for these determinations makes it unnecessary to introduce other manipulations.

The interference of iron in the remaining heavy metal determinations is eliminated by the dithizone separation, in which ferric iron remains quantitatively in the aqueous phase. Iron removal by precipitation reactions is accompanied by heavy metal occlusion and is, therefore, avoided.

Although other reducing agents are often used in the molybdate method for phosphorus (*1*, *31*) the one selected (aminonaphtholsulfonic acid) is rapid and is stable in solution for 2 weeks.

Many extensive discussions of dithizone techniques and principles are to be found in the literature (*3*, *7*, *14*, *15*, *26*, *27*). Both carbon tetrachloride and chloroform are used as solvents for dithizone complexes. The partition characteristics with respect to cobalt (*20*) and other elements make carbon tetrachloride the more satisfactory solvent. Neutral, metal-free carbon tetrachloride is obtained by distillation over calcium oxide. The number and total volume of dithizone–carbon tetrachloride extractions are kept at a minimum by the use of a more concentrated dithizone reagent than is usually advocated. The ammonium citrate buffer used in the alkaline dithizone extraction is best prepared from commercial lead-free citric acid and concentrated ammonium hydroxide and is further purified by dithizone extraction. The completeness of extraction with dithizone may be tested by transferring a small quantity of the green carbon tetrachloride phase to a separatory funnel containing 0.02 *N* ammonium hydroxide, and shaking. The excess dithizone will be extracted into the aqueous phase, and any traces of pink metal-dithizone complexes will be visible in the carbon tetrachloride layer. The appearance of a yellow color in this layer is indicative of decomposition products of the dithizone reagent. These decomposition products do not interfere in the group separations, but will prevent accurate colorimetric measurements, if this reagent is used later in the actual colorimetric determinations.

When the perchloric acid oxidation of the carbon tetrachloride phase of the acid dithizone extraction is carried out as indicated, undesirable tarry residues are avoided.

The amounts of cobalt present in plant tissues are so small that the determination of this element limits the extent to which sample size for the entire procedure can be reduced. If cobalt were not determined, analyses for the remainder of the elements could be made on a much smaller sample of plant material. The Marston and Dewey (*20*) modification of the nitroso R salt cobalt method (*16*) is the most sensitive of the published cobalt methods. The sensitivity of this method is further increased by reduction of final volume and by increased cell depth. Studies on the development of an even more sensitive cobalt method are being carried out at this laboratory.

When copper is determined using sodium diethyldithiocarbamate without a preliminary dithizone extraction, sodium pyrophosphate is added to prevent interference by iron and manganese. The dithizone separation in the present procedure makes this step unnecessary, although ammonium citrate is added to prevent interference by any iron added as an impurity subsequent to the dithizone extraction.

The zinc dithizonate is only moderately stable. The determination should be carried out in a darkened room, and the colors read within an hour. The single extraction does not give complete separation of the dithizone complex. This necessitates careful control in reagent concentration and volume, and in time of shaking to maintain a constant partition ratio (*7*). The final dilution of the complex with carbon tetrachloride is necessary because of the intensity of the colors. The carbon tetrachloride used for dilution must be neutral (as described under Special Precautions and Techniques) or the color complex will be destroyed.

The determination of lead and cadmium on the zinc solution (E) is possible, if desired. Lead is a general contaminant and is present in reagents in quantities exceeding those found in plant tissue, necessitating even more rigorous techniques than herein described.

An attempt was made to develop a titration microprocedure with barium chloride for the determination of sulfur in plant tissues, using tetrahydroxyquinone as an internal indicator. Although this indicator has been used successfully in soil and water analysis, interfering substances were present at every point in the procedure where the method was tried. In using the standard gravimetric barium chloride method for sulfur, it has been found that the citrate remaining in solution C does not interfere and that the traces of sulfur added by the dithizone are not detectable.

It was reported by Smith (*29*) that losses of sulfur occur during wet digestions using perchloric acid. A later publication (*30*) showed that the losses resulted from too vigorous pretreatment with nitric acid and can be avoided. The wet-digestion procedure used has been checked by comparison (in addition to the recoveries reported in Table II) with the A. O. A. C. standard method (*2*), using magnesium nitrate dry-ashing. The results of this comparison, shown in Table IV, indicate that losses of sulfur have not occurred.

In the acid digestions in the preparation of solution F, an aqua regia treatment should precede the perchloric acid digestion to prevent the formation of ammonium salts that

Table IV. Sulfur in Tomato Leaflets

Sample Preparation	Sulfur	Average Value
	Mg./g.	*Mg./g.*
Perchloric acid wet digestion	4.16 4.21	4.18
$Mg(NO_3)_2$ dry ash, 550° C.	4.04 3.96	4.00
Referee analyst: $Mg(NO_3)_2$ dry ash, 550° C.	4.00 3.80 3.76 3.85	3.85

are difficult to decompose. Further aqua regia treatments are necessary to remove traces of residual ammonia, which interfere in the potassium determination. Tests with Nessler's reagent show complete removal of ammonia at this point.

In the ammonium precipitation of iron (aliquot from solution F) for the calcium determination, the bromine oxidation of manganese necessary in soil analysis (*22*) has been omitted, although the quinolate method determines both magnesium and manganese. The traces of manganese present in plant tissue do not ordinarily introduce a detectable error in the magnesium determination. In cases where manganese is exceptionally high, the magnesium values should be corrected.

The centrifuge tube methods of microtitration for calcium and quinolate precipitation of magnesium are used as proposed for the determination of exchangeable bases in soils (*22*) and have been found to be directly applicable.

Wander's recent modification (*32*) of the cobaltinitrite precipitation method for potassium has been found to be both rapid and sufficiently accurate. Blank values should not be subtracted, since the dichromate reduction is not directly proportional to the potassium content below the lower limit specified (Table I). The upper limit may be extended, when a sample of very high potassium content is encountered, by doubling the amount of dichromate, sulfuric acid, and final volume.

The schematic procedure is described (section on Analytical Procedure) in terms of a single determination for each element on the one 7.5-gram sample of plant tissue. As the scheme of analysis is used in this laboratory, duplicate determinations are made of each element. This duplication is accomplished either by dividing the solution from a 15-gram sample after the hydrofluoric acid digestion, or if platinum supply is not limiting, by beginning with duplicate 7.5-gram plant samples. When a 15-gram sample is digested, the specified sizes of apparatus are adequate. The concentration of solution A is kept unchanged by diluting to 250 ml. with 0.6 *N* acid. All subsequent aliquots and volumes are then as indicated. Each run usually consists of 24 samples in duplicate, or a total of 576 individual determinations in addition to standards for the determinations.

This system of analysis has been used successfully with turnip greens, oat straw, oat grain, alfalfa, ladino clover, tomato leaflets, cauliflower, and sugar beet leaves. Although these materials represent a fairly wide range of plant composition, further testing will be required to reveal the full extent of the applicability of the proposed system of analysis.

There are obvious advantages in the application of the proposed analytical scheme to both field and greenhouse investigations involving plant tissue analysis. The techniques described make possible an extensive mineral analysis, using samples of limited size. Another advantage of this type of analytical procedure is its flexibility. The nature of the operations used makes relatively simple the addition of still other elements at appropriate points or the deletion of any determinations not required. Even where different elements are required, and other interfering substances occur, the development of appropriate separations and determinations should be possible.

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Identification of Organic Bases by Means of the Optical Properties of Dilituratе (Nitrobarbiturates)

Aliphatic Amines

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DILITURIC acid (5-nitrobarbituric acid) has been suggested as a satisfactory reagent for the separation of potassium and sodium (*7, 9*) and for the isolation and determination of many organic and inorganic bases (*15*). Fredholm (*9*) determined the ratios of solubilities of alkali and alkaline earth dilituratе and presented photomicrographs of several of them. Chamot and Mason (*6*) also gave photomicrographs of dilituratе of inorganic bases and reported the use of the acid in identifying the bases by the appearance of the crystals developed. Larsen (*13*) determined the optical properties of dilituratе of amino acids as a means of their identification.

Dilituric acid can be prepared by the direct nitration of barbituric acid (*1, 9–12, 17*) and by other methods (*1–4, 16*). The acid is easily purified (*9, 11*).

This investigation was undertaken to determine whether it was possible to use the optical constants of dilituratе to identify the organic bases. Melting-point determinations of the dilituratе are of no value because the compounds decompose upon heating.

Preparation of the Dilituratе

Equivalent quantities of dilituric acid (from Eastman Kodak Company) and the amine were dissolved in a minimum of boiling water and the solution was allowed to cool (*15*). The salts possess high-temperature coefficients of solubility which allow purification by recrystallization. Each dilituratе was recrystallized one or more times.

Analysis of the Dilituratе

Dilituric acid and the dilituratе were dried to constant weight in a vacuum oven at 80° and analyzed for nitrogen by the Kjeldahl method modified to include nitro compounds. The deviation of the percentages found from those calculated did not exceed 0.20 except for the following salts: 2-aminobutane, calculated 22.76, found 22.52; propylenediamine, calculated 26.98, found 26.67; α-methylhydroxylamine, calculated 25.45, found 25.78; hydroxylamine, calculated 27.18, found 26.84; 2,4-diaminobutane, calculated 25.80, found 25.40. The results for these five were obtained with the addition of glucose as a reducing agent (*8*). The salts of ethanolamine and hydrazine were analyzed by the semimicro-Dumas method (*14*). The results for ethanolamine checked closely with the theoretical; hydrazine dilituratе, calculated 34.14, found 34.50.

Determination of Optical Properties

The optical crystallographic data presented in Table I were determined by methods described by Chamot and Mason (*5*) and by Winchell (*18*). The highest index of refraction in the authors' set of liquids was 1.785; hence no value above this figure is reported.

The highest index of refraction for isobutylamine dilituratе could not be determined because of the difficulty experienced in orienting the crystals properly. Hydroxylamine dilituratе possesses very weak dispersion; hence this property was not obtained. Ammonium dilituratе does not show a true value for any principal index in the usual orientations, but the values can be determined from crushed crystals. The dilituratе of *n*-butylamine, ethanolamine, ethylamine, ethylenediamine, methylamine, α-methylhydroxylamine, and isopropylamine show but one index, β, in the usual orientation. All the other compounds present two true indices in the most frequent orientations. All extinction angles were measured in the acute angle, β.

In Table II are recorded some optical and crystallographic values which were observed on the most frequently occurring orientations of the crystals. Most of the dilituratе of the primary aliphatic amines are so flattened that the crystals tend to assume a common orientation when suspended in an immersion liquid. The optical orientation designated as acute, obtuse, or optic normal indicates that a centered interference figure is found in the usual position of the crystals. The descriptive term "inclined" indicates that the interference figure is not centered. In these inclined obtuse orientations one of the optic axes usually presents itself, but the view of the crystal is essentially on the obtuse bisectrix side. Thick crystals of ammonium dilituratе present no definite orientation, hence "variable" optical orientation for

Table I. Optical Properties of Some Dilituratе of Primary Amines and of Dilituric Acid

Dilituratе	Extinction Angle	Optic Sign	Refractive Indices Alpha	Beta	Gamma	Elongation	Dispersion
Dilituric acid	31	−	1.388	1.684	> 1.785	−	v > ρ
2-Aminobutane	Parallel	−	1.452	1.650	1.662	+	ρ > v
2-Amino-*n*-octane	30	−	1.469	1.635	1.647	±	ρ > v
Ammonium	44	−	1.427	1.772	> 1.785	±	v > ρ
Isoamylamine	6	−	1.456	1.644	1.672	±	v > ρ
n-Amylamine	Parallel	+	1.596	1.614	1.679	+	v > ρ
Benzylamine	Parallel	−	1.448	1.766	> 1.785	−	v > ρ
Isobutylamine	41	−	1.470	1.706	...	−	None
n-Butylamine	43	−	1.476	1.704	1.783	+	v > ρ
Cyclohexylamine	29	−	1.483	1.648	1.697	±	v > ρ
2,4-Diaminobutane	39	−	1.455	1.761	> 1.785	−	v > ρ
Diethylenetriamine	26	−	1.446	1.731	1.736	±	v > ρ
Ethanolamine	39	−	1.458	1.744	1.777	−	v > ρ
Ethylamine	43	−	1.459	1.734	> 1.785	−	v > ρ
Ethylenediamine	44	−	1.454	> 1.785	> 1.785	−	v > ρ
n-Heptylamine	Parallel	+	1.592	1.616	1.660	−	v > ρ
Hydrazine	26	−	1.458	1.748	1.783	−	v > ρ
Hydroxylamine	25	−	1.487	1.748	1.763	−	...
Methylamine	31	−	1.426	1.696	> 1.785	−	v > ρ
α-Methylhydroxylamine	38	−	1.416	1.702	> 1.785	±	v > ρ
Isopropylamine	43	−	1.447	1.701	1.782	±	v > ρ
n-Propylamine	Parallel	+	1.606	1.619	1.701	−	v > ρ
Propylenediamine	28	−	1.452	> 1.785	> 1.785	±	v > ρ

Table II. Apparent Properties of Primary Aliphatic Amine Dilituratesfrom Most Frequently Observed Orientation

Dilituratе	System	Habit	Optical Orientation	Extinction Angle	Refractive Indices	
Dilituric acid	Monoclinic	Tabular	Obtuse	Parallel	1.388	1.684
2-Aminobutane	Orthorhombic	Acicular	Optic normal	Parallel	1.452	1.662
2-Amino-*n*-octane	Monoclinic	Foliated	Obtuse	6	1.469	1.635
Ammonium	Monoclinic	Equant	Variable	Variable	Variable	Variable
		Tabular	Inclined obtuse	0–15	Variable	> 1.785
Isoamylamine	Monoclinic	Acicular	Acute	6	1.644	1.672
n-Amylamine	Orthorhombic	Foliated	Optic normal	Parallel	1.596	1.679
Benzylamine	Orthorhombic	Tabular	Optic normal	Parallel	1.448	>1.785
Isobutylamine	Monoclinic	Foliated	Obtuse	14	1.470	1.706
n-Butylamine	Monoclinic	Tabular	Inclined obtuse	Parallel	Variable	1.704
Cyclohexylamine	Monoclinic	Acicular	Obtuse	Parallel	1.483	1.648
2,4-Diaminobutane	Monoclinic	Tabular	Optic normal	20	1.455	> 1.785
		Fibrous		20–30		
Diethylenetriamine	Monoclinic	Lamellar	Optic normal	Parallel	1.446	1.731
Ethanolamine	Monoclinic	Lath-shaped	Inclined obtuse	39	Variable	1.744
Ethylamine	Monoclinic	Lath-shaped	Inclined obtuse	Parallel	Variable	1.734
Ethylenediamine	Monoclinic	Tabular	Inclined obtuse	44	Variable	> 1.785
n-Heptylamine	Orthorhombic	Lamellar	Optic normal	Parallel	1.592	1.660
Hydrazine	Monoclinic	Columnar	Obtuse	24	1.458	1.748
Hydroxylamine	Monoclinic	Tabular	Optic normal	25	1.487	1.763
Methylamine	Monoclinic	Lamellar	Inclined obtuse	Parallel	Variable	1.696
α-Methylhydroxylamine	Monoclinic	Tabular	Inclined obtuse	38	Variable	1.702
		Lamellar	Optic axis	..	...	1.702
Isopropylamine	Monoclinic	Tabular	Inclined obtuse	43	Variable	1.701
n-Propylamine	Orthorhombic	Lamellar	Optic normal	Parallel	1.606	1.701
Propylenediamine	Monoclinic	Lamellar	Obtuse	21	1.452	> 1.785

those which possess equant habit. Tabular crystals of the ammonium compound show an inclined obtuse bisectrix figure. Apparent refractive indices of some of the compounds could not be satisfactorily determined within narrow limits and are therefore reported as variable. These variable indices of all compounds besides equant ammonium are greater than the true index α, but less than the true index β. Crystal habit is described according to Winchell (*18*).

In Figures 1 and 2 are diagrams of the crystals as they appear in the most frequently occurring orientations. Observed refractive indices (in the direction of the dotted lines) and observed crystal angles are recorded on most of

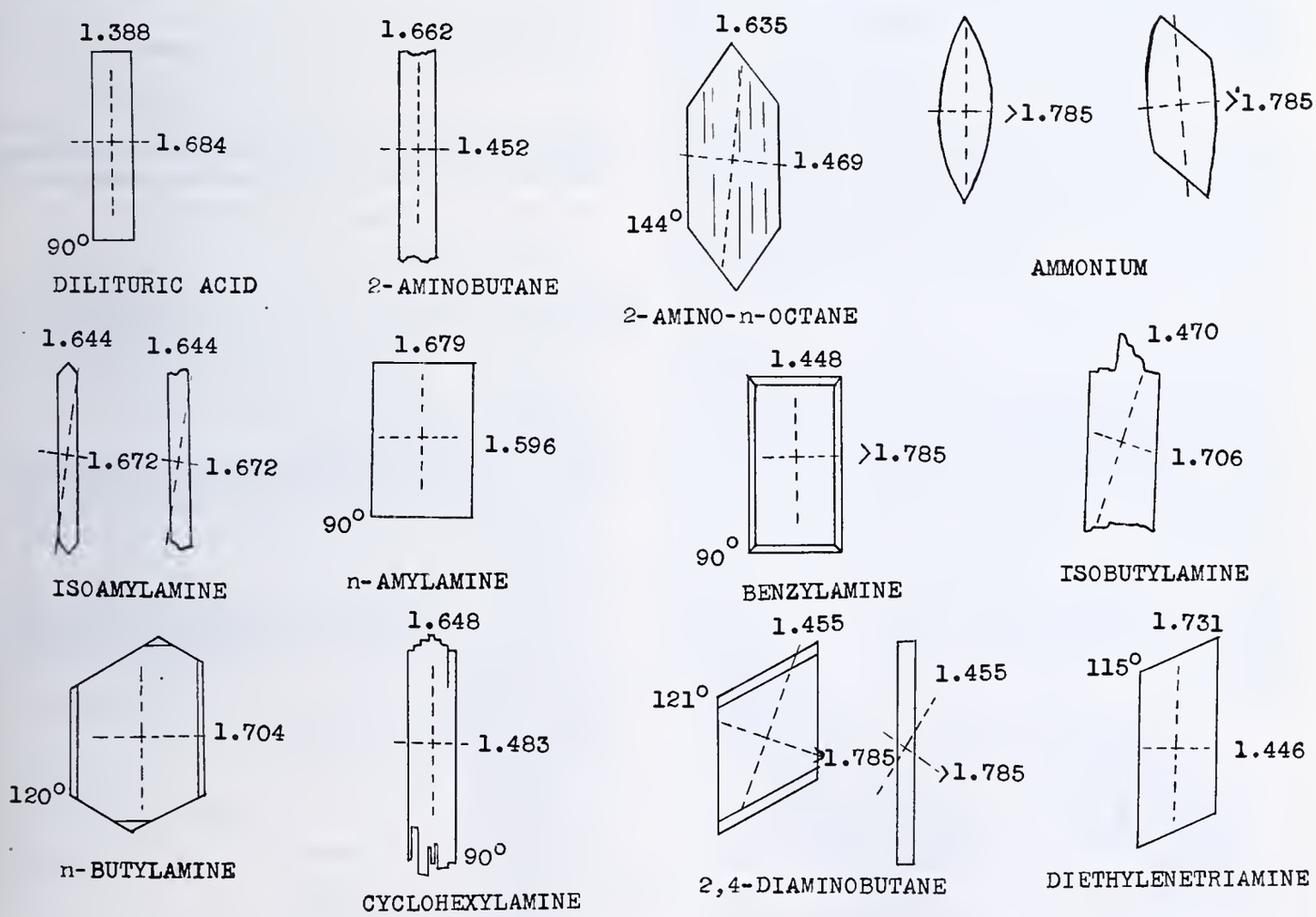

Figure 1. Diagrams of Crystals of Amine Dilituratesfrom

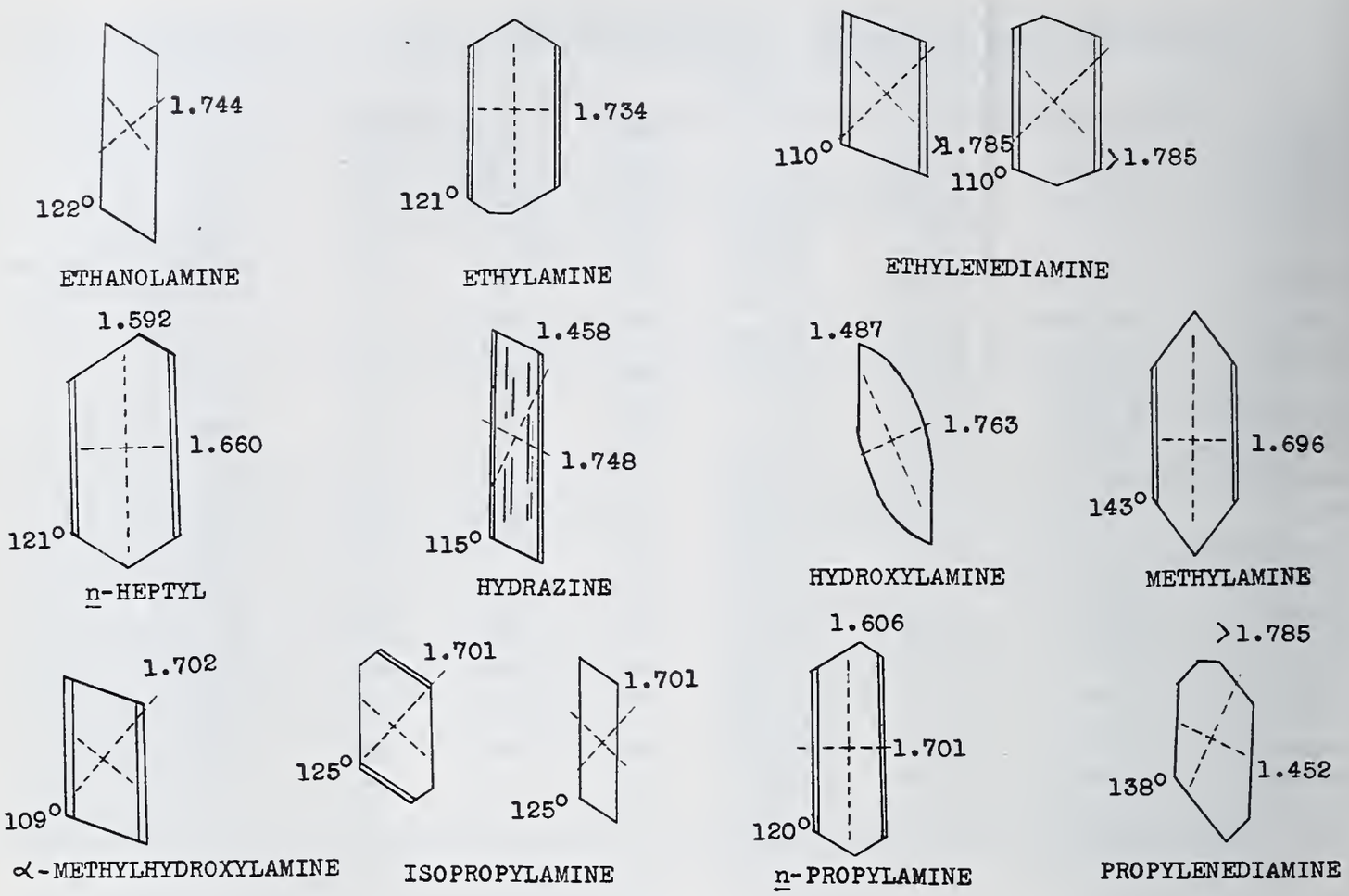

Figure 2. Diagrams of Crystals of Amine Dilturates

the diagrams. The angle for α-methylhydroxylamine diliturate varies considerably; a value as high as 131° is frequently noted on lamellar crystals, whereas 109° is a good average for tabular crystals. Crystal angles for diliturates of propylenediamine and isopropylamine also vary a few degrees either way from those values recorded for each. Diliturates of 2-aminobutane, isobutylamine, and isoamylamine present irregularly shaped or broken ends. Occasionally the latter compound shows angles of approximately 138°. Cyclohexylamine diliturate possesses irregularly shaped ends, but 90° steps are usually noted.

Hydroxylamine diliturate crystals are characteristically boat-shaped. Sometimes ammonium diliturate crystals are boat-shaped, but can easily be distinguished from hydroxylamine diliturate by means of the various constants given.

Diethylenetriamine diliturate shows a definite cross-patching pattern or cleavage lines, especially in the thicker crystals. Hydrazine diliturate shows decided cleavage. The diliturate of 2-amino-*n*-octane exhibits slight cleavage. This compound occurs as masses of crystals which must be crushed to isolate individuals.

Binary mixtures of amines (methyl and ethyl, methyl and *n*-propyl, methyl and *n*-amyl, isobutyl and *n*-butyl, ethyl and *n*-propyl, *n*-propyl and *n*-butyl) in equal proportions were used in preparing diliturates. The resulting compounds of the first four mixtures were composed of two types of crystals, each possessing the same optical properties as those exhibited when prepared from the pure individual amine; whereas the last two mixtures presented many crystals with properties other than those determined from the pure amines.

These compounds have been successfully used as unknowns for students in an elementary chemical microscopy course.

Summary

The optical crystallographic data for 22 primary aliphatic amine diliturates and for dilituric acid have been determined. The optical properties provide a means of identifying pure amines and some mixtures of amines.

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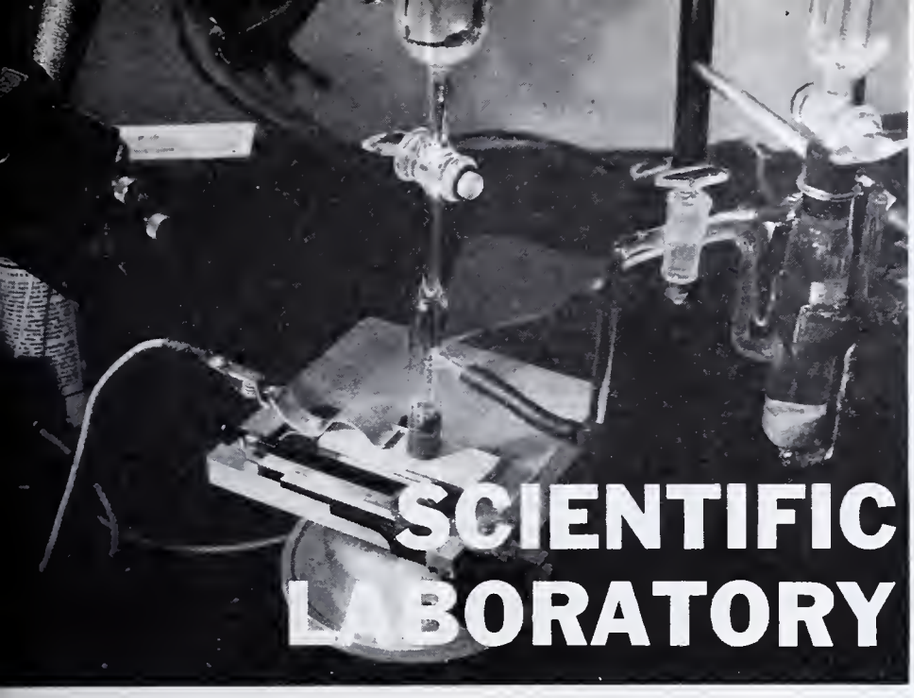


SCIENTIFIC APPARATUS LABORATORY EQUIPMENT

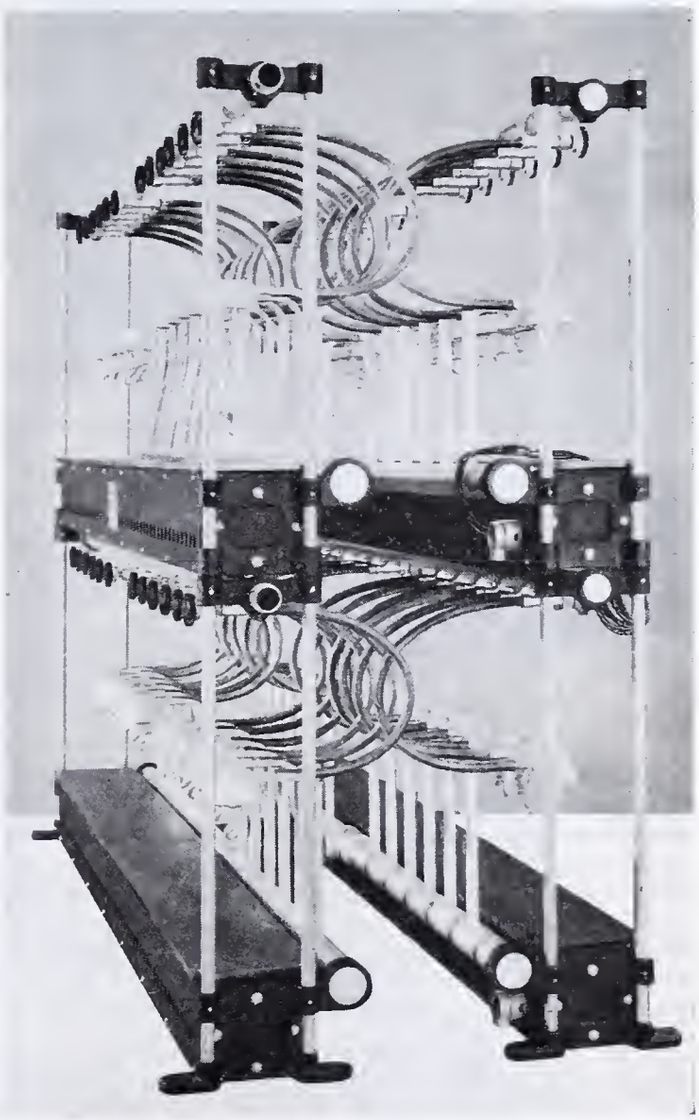
Multiple-Extraction Rack for Chemical Analysis of Rubber Products

Utilizing extraction condensers, as specified in A. S. T. M. Designation D297-41T for the chemical analysis of rubber products, the multiple unit electrically heated extraction racks developed by the Precision Scientific Co., Chicago, are especially applicable to routine extractions incident to the production of synthetic rubber.

Faced with the difficult problem of installing 192 extraction units in one laboratory where space was none too plentiful, the technicians of an important producer of synthetic rubber found no practical solution until the design, exemplified by the accompanying photographs, was submitted.

The multiple assembly, consisting of 48 extraction units, is composed of two sets of 24 units each, arranged back-to-back; each set is composed of two banks of 12 units each, one deck above the other. The 48-unit assembly is only 48 inches long, 24 inches wide, and 39 inches high over-all. If placed on a table 36 inches high, all units are at a comfortable height to facilitate handling and observation.

Water supply and discharge manifolds serve the extraction condensers, the water supply to each condenser being controlled by a needle valve. Condensers conform to A. S. T. M. Method



D297-41T. They can be used with 250- or 400-ml. flasks, and are spaced on 3.375-inch centers.

To permit elevation of each condenser for placement or removal of the flask, the condenser overflow pipe telescopes into a vertical pipe communicating with the water discharge manifold, while the connection between condenser inlet and water supply valve is flexible synthetic rubber tubing.

Electrically heated hot plate tops are heat-treated heavy cast iron, with strip units fastened to the underside, and the remaining space within the hot plate housing is packed with thermal insulating material to promote heat efficiency. Each hot plate includes a 3-heat switch.

Multisource Unit

A new electrical source unit for spectrographic analysis has been announced by the Harry W. Dietert Co., Detroit, Mich., and its associate, the Applied Research Laboratories, Glendale, Calif. The unit is said to combine the functions of the conventional direct current arc, alternating current arc, and high-voltage condensed spark units, and to go beyond them in providing precisely controlled excitation suitable for all types of analysis.

With this unit the three factors of an electrical circuit—resistance, inductance, and capacitance—may be set and precisely controlled to selected values over wide ranges. By virtue of the wide variation in excitation conditions obtainable, either very arclike or sparklike spectra, with all variations in between, may be had. The fine adjustment and control of the discharge afforded by the adjustable resistance, inductance, and capacitance mean that the Multisource excitation combines the sensitivity of the arc with the accuracy of the spark.

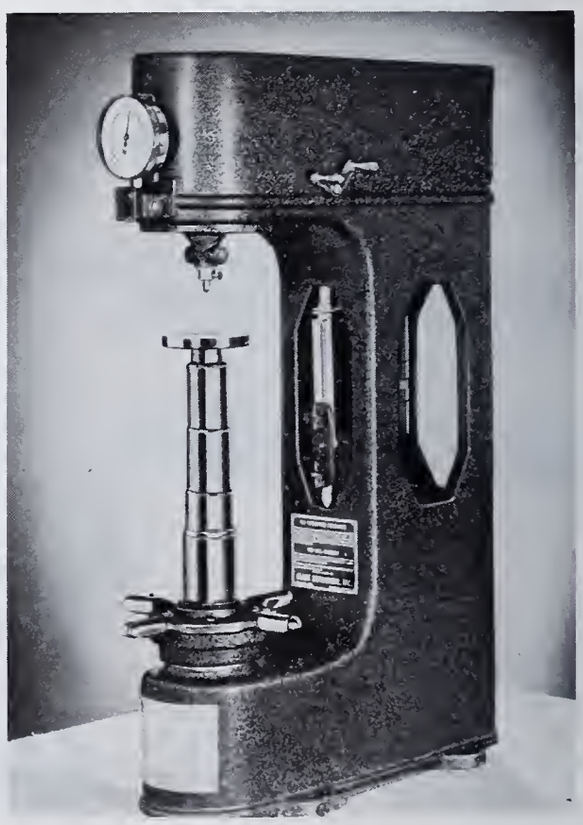
The unit consists of a main control panel mounted at the front, and a selector panel mounted at the side and equipped with pull knobs which allow selection of the conditions of discharge. Seven knobs at the front of the condenser section allow the selection of capacitance values from 1 to 60 microfarads in steps of 1 microfarad. Ten knobs allow selection of resistance values from 1 to 400 ohms in steps of 1 ohm, while five knobs on the inductance section allow values from 25 to 400 microhenrys to be utilized. This switching is done behind the panels by means of heavy well-insulated knife switches to assure certainty of contact and freedom from trouble.

The main panel is supplied with an oscillograph, so that the current, duration, and wave form of an average individual discharge can be studied. This is useful as a means of predicting the properties of a particular discharge and for adjusting the phase of the charging cycle with respect to that of the discharge. An ammeter is furnished to check the power consumption of the unit, and a voltmeter to check the consistence of the voltage across the condensers before discharge. Another voltmeter indicates the difference between the input voltage and regulated voltage, so that the instrument can be adjusted to take into account extremes of line voltage variations, preventing decrease in accuracy on this account. A phase control knob, coupled directly to the synchronous gap, provides the necessary adjustment of the ignition point of the discharge. An adjustable 0- to 2-minute synchronous timer allows the total time of operation to be precisely controlled.

Hardness Tester

Clark Instrument, Inc., Dearborn, Mich., announces an improved hardness tester for Rockwell testing in three models, US8, US12, and US16. The instrument is said to embody a number of features contributing to accuracy of measurement and speed of operation. Among these are a frictionless spindle which provides a correct minor load at all times, zero drag trip which eliminates friction or drag on the loading beam and ensures consistent accuracy of the major load, a dial indicator adjustment affording a convenient, simple means of maintaining accuracy of readings, and a minor load adjustment.

Other features are fully enclosed elevating screw with self-lubricating oil reservoir, and an adjustable steady rest. The instrument is supplied complete with diamond cone penetrator and steel ball penetrators for Rockwell testing on C, B, and most other standard Rockwell scales.



Meter for Checking Distilled Water

A new instrument which provides a practical and quick way to check the purity of distilled water has been introduced by the Barnstead Still and Sterilizer Co., Boston, Mass. Called the Barnstead purity meter, this instrument is easy to use.

To make a purity check of distilled water, the temperature of the water is taken first. Next, a dial on the purity meter is set to the corresponding temperature. Then, with the conductivity cell immersed in the distilled water, a dial is moved until the electric radio eye indicates the correct setting. At this point, the dial indicates the impurity content of the distilled water in terms of parts per million as sodium chloride. The whole procedure takes but a few seconds and gives a quick check-test against a predetermined standard.

The meter is a compact conductivity bridge, 6.75 × 5 × 2.625 inches, and it weighs only 3.25 pounds. It requires no batteries, but connects directly to 110-volt 60-cycle outlet. Measurement scale reads from 0 to 15 p. p. m. as sodium chloride. Temperature correction scale 64° to 190° F.

• • • • • • •

New Laboratory Furnaces

A line of laboratory furnaces, new in appearance and construction, has been developed by the Lindberg Engineering Co., 2444 West Hubbard St., Chicago 12, Ill. The line includes the box furnace for drying precipitates, ash determinations, fusions, ignitions, etc.; the combustion tube for carbon determination, standard combustion, and organic analyses; the crucible furnace for melting base metals, thermocouple calibrations, molten salt baths, etc.; and hot plates for heating, distilling, boiling, evaporating, etc.

The new furnaces are patterned, in many respects, after the company's production furnaces. They incorporate such features as the convenient door operating mechanism on the box furnace, which permits cooler working conditions; low-voltage, high-temperature type heating elements; the use of a Lindberg input control for smooth, stepless apportioning of heat; and the streamlined shape which matches other modern laboratory equipment and contributes to the high standard of laboratory cleanliness. The furnaces are available in different sizes for operation on 110- or 220-volt alternating direct current.

BOOK REVIEWS

Manual of Laboratory Glass-Blowing. *R. H. Wright.* 90 pages. Chemical Publishing Co., Brooklyn, N. Y. Price, $2.50.

This manual of glass blowing is excellently presented, with photographic illustrations, and is worthy of study and use by those interested in this subject.

The following suggestions are offered:

The Torch (page 18, paragraph 2). The torch should be so mounted that it can be readily pulled out away from the bench. It is highly desirable that the glass blower sit or stand sideways to the bench, which should be at his right. This allows greater freedom of movement and should be "an absolute must", especially in the fabrication of apparatus to be used in research laboratories. Where natural gas must be used a very serious problem is presented, in that this gas blows out very easily, even when oxygen is used. This trouble can be eliminated by tapping a hole in the gas feed of the torch and screwing in a short section of copper tubing 0.25 inch in diameter, to the other end of which has been attached a tiny Bunsen burner. This is then bent into place just under the jet of the torch. A tiny flame is sufficient to keep the torch from blowing out, regardless of how fast a flow of oxygen is used. A valve is necessary in the Bunsen burner to control the size of the flame.

The Blowing Tube (page 20, paragraph 1). The blowing tube is a very useful and necessary gadget, in its place. It is necessary to use it when the apparatus being worked on is too long, cumbersome, or stationary for the glass blower to bring it to his mouth to blow. All other types should be handled without it. Beginners especially should avoid its use on smaller articles, because a blow tube here hampers rather than helps the proper manipulation of the work.

Cutting Files (page 20, paragraph 2). A good file for cutting Pyrex is the 8-inch mill file, second cut. This file has four cutting edges, which can be ground and sharpened on an emery wheel over and over again.

Joining Tubes of Equal Size (page 23, Chapter IV). When the ends of two pieces to be joined have been properly heated and pressed together (page 24, paragraph 2) they should be immediately pulled apart somewhat, so that the glass squashed together will not be too thick. This is very helpful, whether the job is finished by spotting it together—that is, heating one spot at a time—or whether it is rotated in the flame and heated all around the tube and then blown in and out several times. In this operation the beginner should avoid use of the blow tube. It is better to learn without it. Wherever the blow tube must be used, it should be attached to the tube or part held in the left hand. The heaviest, longest, or bulkiest part of any apparatus being worked on in the torch should be held in the left hand. The left hand should be held above the work. Figure 1, Plate 2, is a good example. Here the right hand is wrong. The right hand should be under the work and the tube held and rotated round and round or back and forth, between the thumb and first two or three fingers in unison with the left hand. It takes practice to master this procedure, but better results make the effort worth while.

(Page 32, paragraph 1, last sentence.) In this operation the glass should never be "cut" with a file or knife. These tools leave a "white" mark on the edge of the glass which is still there after the two tubes have been joined together. It is especially important to eliminate these marks in vacuum apparatus, where they can easily cause trouble. For instance, if a leak is suspected somewhere in the apparatus and an effort is made to find it with a high-frequency coil (see page 75), another leak might be caused by the attraction that this white mark has for the spark from the coil. To avoid this the end of the tube—in fact, all pieces of glass that are to be joined or sealed together—should be blown open in the fire, never cut with file or knife.

Flaring the End of a Tube (page 55, bottom). In this operation a reamer made of brass is best to use. The thickness is not important; $^1/_{16}$ inch is good. In shape it is like half an elongated diamond, roughly 3 or 4 inches long, and about 1.5 or 2 inches at its wide part. From the middle of its wide part an end projects for 2 inches or so, to which is attached a wooden handle to insulate against heat. To prevent the hot glass from sticking to the reamer, it should be moved across a piece of beeswax while warm.

It is not overly clear how the mathematics involved in the latter part of the book are helpful in glass blowing.

Frank L. Hayes

Chemical Analyses of the Waters of Oklahoma

"Chemical Analyses of the Waters of Oklahoma" is the title of a 474-page book written by O. M. Smith. and published as Bulletin 52 of the Engineering Experiment Station, Oklahoma Agricultural and Mechanical College, Stillwater, Okla. Considerably more than half of the volume consists of tables of analyses of city supplies, wells, lakes, ponds, and streams.

This is a compilation that represents the joint effort of workers at the Oklahoma Agricultural and Mechanical College, the Oklahoma State Health Department, and the Oklahoma Geological Survey, over several years. Robert H. Dott, director of the Oklahoma Geological Survey, and E. C. Warkentin, assistant state sanitary engineer of the Oklahoma State Health Department, are contributors.

Priorities on Laboratory Materials

Priority assistance for materials required by scientific and technological laboratories and deliveries of laboratory equipment are affected by two amended orders (P-43 and L-144) issued June 26 by the War Production Board.

Preference Rating Order P-43 as amended assigns preference ratings and an allotment symbol to assist laboratories in obtaining materials and equipment needed to carry on scientific or technological activities.

All laboratories receive a preference rating and allotment symbol under the amended order; laboratories carrying on work of great importance to the war effort are entitled to obtain a serial number and may use a higher preference rating than other laboratories. All laboratories owned or operated by the Army or Navy receive high preference ratings.

L-144 as amended restricts the delivery of any one item or quantity of the same item of laboratory equipment having a value of $50 or more, or delivery on a purchase order of laboratory equipment worth $200 or more, even though no item or quantity of the same item on the order has a value of $50 or more, unless authorized by the War Production Board on Form WPB-1414 (formerly PD-620).

The order defines "laboratory equipment" to mean any apparatus, instrument, or the like, designed primarily for use in laboratories.

Purchase orders of the Army and Navy, and laboratories which have obtained serial numbers under Preference Rating Order P-43 as amended, are exempted from this restriction, except on purchase orders for certain highly critical equipment named on List A attached to the order.

Interpretation I to L-144, issued July 17, states that the same regulations governing delivery to domestic sources apply to deliveries for shipment to foreign firms or individuals.

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The Mass Spectrometer as an Analytical Tool

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Discussing the mass spectrometer as an analytical tool, the paper describes the method of analysis, presents results obtained with this method, and gives information regarding the commercial instrument that is now in use.

THE demands upon the petroleum and chemical industries for large quantities of aviation gasoline, synthetic rubber, and other war materials have resulted in employing many new processes on a large scale. These new processes in general are more complex than those formerly employed in industry. To develop such new processes and to put them into operation necessitates a large amount of preliminary and coordinated analytical work.

These technological advances have therefore required a parallel advance in the art of analysis both as an aid to the development of new processes and for the analytical control of the plants after they have been put into operation.

One of the new methods of analysis which are now being employed is the mass spectrometer method. It is the purpose of this paper to discuss the mass spectrometer as an analytical tool: the method of analysis with the mass spectrometer, a few results obtained with this method, and the commercial instrument now in use.

Principle of Operation of Mass Spectrometer

In general, a mass spectrometer is a device for sorting molecules. Figure 1 is a diagrammatic sketch of the Dempster or 180° type of mass spectrometer. The gas mixture to be analyzed enters through the gas inlet to chamber *a*.

Before the molecules are sorted, they are given an electric charge, so that they can be forced to move by the combined action of electric and magnetic fields. The production of an electric charge on a molecule, or ionization, is accomplished by bombarding the molecules with a stream of electrons in an ionization chamber. In practice the energy in the bombarding electrons is usually made sufficiently high to break the molecules into charged fragments. This feature enables substances of the same molecular weight, but of different molecular structure, to be distinguished from each other (*2*).

The ionization chamber and associated electron source or filament are shown schematically in Figure 1 at *a*. The ions are pulled out of chamber *a* by means of the electric field existing between electrodes *d*, *e*, and *f*. Ions enter the analyzer tube with a high velocity and are sorted out according to their mass by the action of a magnetic field. The magnetic field causes heavy ions to follow a circular path which is of greater radius than the path followed by lighter ions. The radius of the path for an ion of given mass can be made smaller or larger by varying the electric field produced between electrodes *d*, *e*, and *f*. Therefore, by gradually varying the electric field, ions of each mass can be caused to fall successively on the collector, *c*, where their quantity is measured by amplifying and recording equipment. The resulting record shows the relative numbers of ions of each mass which appear at *c*.

Mass Spectra

Figure 2 shows the automatically recorded mass spectra of *n*-butane and of a paraffin-olefin mixture, C_2 through C_4. It takes about 10 minutes to make a single record. When operating in a routine manner a sample can be run every 20 minutes.

In order to make the records more easily understood it is necessary to explain that there are four traces, all recorded simultaneously and having different degrees of sensitivity. The top trace has unit sensitivity, the second has a sensitivity of one third, the third a sensitivity of one tenth, and the fourth or bottom trace a

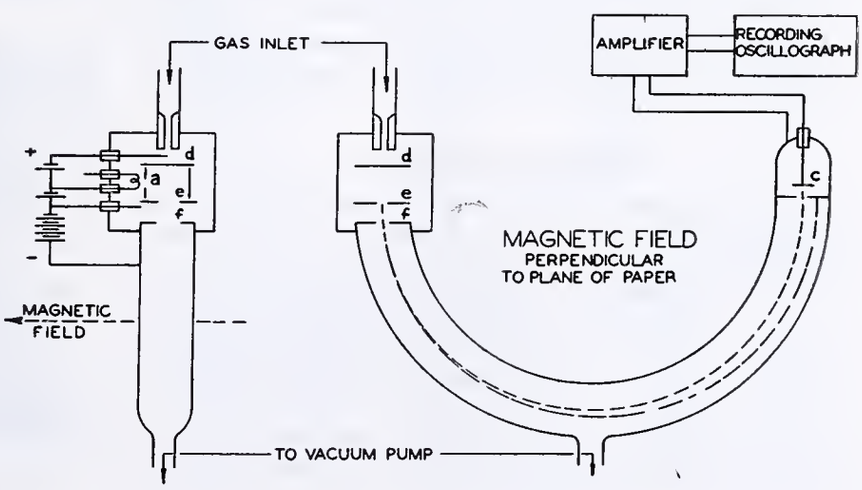

FIGURE 1. DIAGRAM OF MASS SPECTROMETER

TABLE I. ANALYSIS OF DEPROPANIZER OVERHEAD

Mass	M, Mixture Peaks	n-Butane	Iso-butane	Propane	Ethane	Methane	Σ, Sum of Component Spectra	$M - \Sigma$, Residuals
15	167.3	1.0	1.6	30.4	23.0	110.3	166.3	+1.0
16	134.4	0.0	0.0	0.9	0.8	132.7	134.4	0.0
26	146.1	1.6	0.9	37.6	106.4	...	146.5	−0.4
27	361.6	10.8	12.1	183.0	158.0	...	363.9	−2.3
28	777.8	9.3	1.2	275.3	494.4	...	780.2	−2.4
29	593.1	12.5	2.6	467.8	105.6	...	588.5	+4.6
30	130.0	0.3	0.0	10.0	119.7	...	130	0.0
31	2.4				2.5	...	2.5	−0.1
38	24.0	0.5	1.2	21.9	...	...	23.6	+0.4
39	98.0	4.3	8.7	84.6	...	...	97.6	+0.4
40	14.7	0.6	1.3	13.0	...	...	14.9	−0.2
41	94.1	9.0	18.9	65.8	...	...	93.7	+0.4
42	49.9	3.9	15.6	30.2	...	...	49.7	+0.2
43	207.4	32.2	49.8	124.5	...	...	206.5	+0.9
44	145.3	1.0	1.6	142.7	...	...	145.3	0.0
45	4.6	0.0		4.6	...	...	4.6	0.0
50	0.6	0.3	0.3	...	...	...	0.6	0.0
51	0.5	0.3	0.3	...	...	...	0.6	−0.1
52	0.1	0.1	0.1	...	...	...	0.2	−0.1
53	0.6	0.3	0.3	...	...	...	0.6	0.0
54	0.1	0.1	0.0	...	...	...	0.1	0.0
55	0.7	0.3	0.2	...	...	...	0.5	+0.2
56	0.5	0.3	0.2	...	...	...	0.5	0.0
57	2.5	0.9	1.6	...	...	...	2.5	0.0
58	5.0	3.8	1.2	...	...	...	5.0	0.0
59	0.2	0.2	0.0	...	...	...	0.2	0.0

Computation of % of n- and isobutane — *Mole %*

From peak 57: $\underline{0.503}\, p_n + \underline{0.654} p_i = 2.5$ (1)

From peak 58: $\underline{2.10} p_n + \underline{0.498} p_i = 5.0$ (2)

n-Butane	where p_n = % n-butane	= 1.8	
Isobutane	and p_i = % isobutane	= 2.4	
Propane	$(145.3 - 1.0 - 1.60)\ \underline{0.269}$	= 38.4	(3)
Ethane	$(130.0 - 0.3 - 0 - 10.0)\ \underline{0.3175}$	= 38.0	(4)
Methane	$(134.4 - 0 - 0 - 0.9 - 0.8)\ \underline{0.146}$	= 19.4	(5)

Underlined coefficients are obtained from calibrations.

sensitivity of one thirtieth. This device enables the height of any peak to be recorded within better than 1 per cent accuracy over a range in magnitude of 250 to 1.

The abscissas, or horizontal scale values, on the mass spectra at which peaks occur, represent molecular weights corresponding to the different charged fragments obtained when n-butane or a C_2 to C_4 gas mixture is bombarded with an electron stream. The ordinate or vertical scale at each of these masses is a measure of abundance of the particular fragment which is formed.

For any given gas the particular fragments formed depend for the most part upon the number and type of atoms in the molecule. The relative abundance of the different fragments, or pattern of the mass spectrum, depends upon the structure of the molecule.

The mass spectrum, therefore, depends on the structure of the molecule, as well as upon the type of atoms of which the molecule is composed—for example, if this mass spectrum were for isobutane it would have peaks at the same masses but their relative heights would be different. It is this dependence of the mass spectrum upon the structure of the molecule that enables the isomers of a compound to be separately determined.

Principles of Analysis

If now the mass spectrum of an unknown mixture is obtained, employing a suitable technique, it will be a composite mass spectrum and can be considered as being a summation or superposition of the mass spectra of all the components of the mixture. The mass spectrum of the C_2 through C_4 mixture shown is such a composite spectrum. The analysis of the mixture consists of the unraveling of the mixture spectrum. Through methods developed in the Consolidated Engineering Corporation laboratory, this unraveling has been made much simpler than was originally thought possible. One other point of interest here is that all peaks recorded are not generally needed for an analysis. The peaks which are not used for calculating the mixture are used as a check on the accuracy of the analysis. They are employed to indicate calculation errors, instrumental errors, or failure to take account of a gas in a mixture. This final check on an analysis has been found to be very valuable.

In order to complete the analysis of a mixture from its composite spectrum, it is necessary to know the mass spectra of all its components. These spectra are obtained by running pure gases through the instrument and are called calibrations. The record of the n-butane in Figure 2 is an example.

An explanation of the general methods of analysis would involve more discussion than space permits. However, the extremely simple examples shown in Table I will serve to clarify some of the previous remarks.

In the first column are listed the masses or molecular weights at which peaks occur on the mass spectrum of the unknown mixture. In the second column are recorded the peak heights read from the automatic record of the mixture.

The analysis of the mixture may be obtained in the following manner: The only components contributing to peaks at mass 57 and 58 are n- and isobutane. The percentages of n- and isobutane are computed from simultaneous Equations 1 and 2 shown at the bottom of the table. In these equations the underlined numbers are taken from calibration records obtained by running pure n- and isobutane. From the determination of the percentages of n- and isobutane and from their

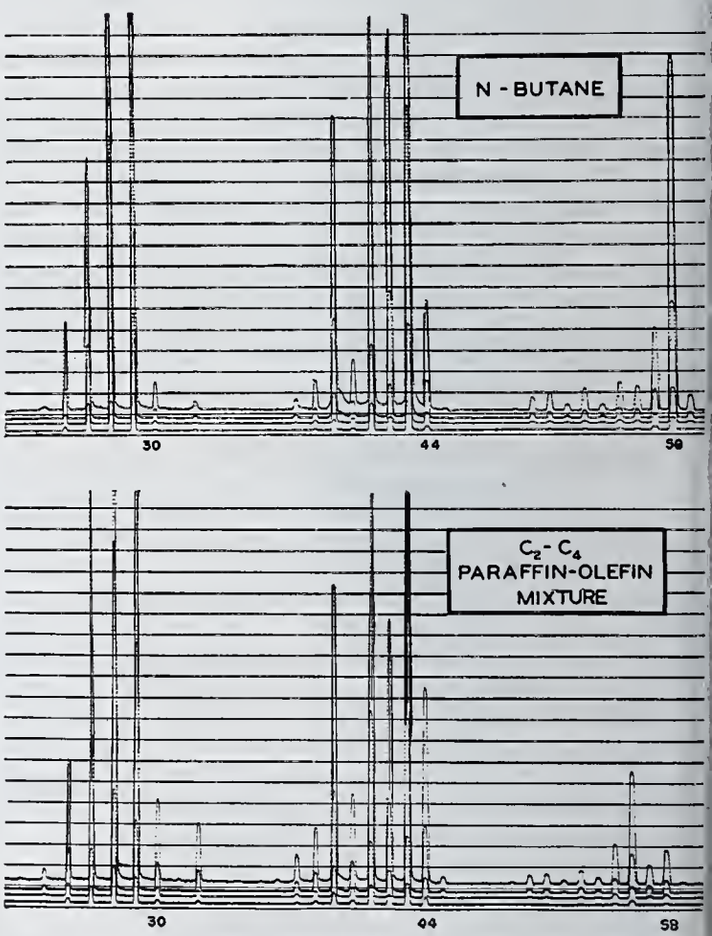

FIGURE 2. AUTOMATICALLY RECORDED MASS SPECTRA

Table II. Wet Gas

	Mass Spectrometer *Mole %*	Fractionating Column *Mole %*
N_2	1.1	...
Methane	84.1	87.44
Ethane	4.9	4.60
Propane	4.7	4.30
Isobutane	1.1	0.72
n-Butane	1.7	1.47
Isopentane	0.7	0.55
n-Pentane	0.5	0.47
C_6^+	0.2	0.45
C_4 naphthenes	0.6	...
C_5 naphthenes	0.3	...
C_6 naphthenes	0.1	...

	Time Required for Analysis by Complete Direct Method (Tables II and III) *Min.*
Mass spectrometer (instrument time)	
Mixture	25
Prorated calibration	4
Taking data from record (technician's time)	
Mixture	20
Prorated calibration	4
Computing	80
Total man-hours	2.25

Table III. Wet Gas

(Two analyses of this mixture on fractionating column A and two on B are compared with mass spectrometer analysis.)

	Fractionating Column A		Fractionating Column B		Mass Spectrometer
	Mole per cent				*Mole %*
H_2	...	...	...	...	0.2
N_2	...	...	...	...	0.4
O_2	...	...	...	...	0.2
CH_4	91.500	91.600	91.517	91.557	89.9
C_2H_6	4.395	4.210	4.372	4.326	4.1
C_3H_8	1.942	1.942	1.968	1.969	2.0
i-C_4H_{10}	0.612	0.583	1.255	1.262	1.0
n-C_4H_{10}	0.619	0.699			0.5
	1.231	1.282			
i-C_5H_{12}	0.290	0.272	0.491	0.488	0.1
n-C_5H_{12}	0.227	0.222			0.2
	0.517	0.494			
C_6	0.232	0.230	0.236	0.230	...
C_7^+	0.149	0.162	0.161	0.168	...
C_3H_6O	...	...	...	...	1.4

calibration spectra, the contributions of *n*- and isobutane to each of the masses listed can be readily computed. These values are shown in the third and fourth columns.

If now the mass 44 contributions of *n*- and isobutane are subtracted from the mixture 44 peak, the remaining peak is due entirely to propane. The per cent propane in the mixture is computed from this remainder peak and from the sensitivity of the 44 peak to propane. This simple calculation is shown in Equation 3 where the underlined value, 0.269, is the sensitivity in per cent per division obtained from the propane calibration.

Similarly, the amount of ethane can be computed by subtracting the 30 peak contributions of the heavier components from the mixture 30 peak. This determination is shown in Equation 4.

The same procedure is used in calculating the amount of methane as indicated in Equation 5.

If the apparatus were perfect, if there were no errors in the computation, and if all constituents in the mixture were taken into account, the sum of these component spectra for any given mass would be equal to the mixture peak. The comparison of the sums of the component spectra for each mass with the mixture peak of the corresponding mass therefore offers an excellent check on the reliability of the analysis.

The sums of the component spectra are shown in column 8. In order more clearly to show the agreement of this summation column with the mixture column, the difference between these two columns is shown in column 9. Because of the method of computation, the residuals will be zero on the masses used for computing the mixture—viz., 16, 30, 44, 57, 58. The residuals on the peaks not used for determining the analysis of the mixture are a measure of the accuracy of the analysis. In this case all residuals are less than 1 per cent of the respective mixture peaks. This shows that the analysis is of good accuracy and that all substances present in detectable amounts have been accounted for.

The ability of this method to detect unexpected components is illustrated in Tables II and III. The unexpected naphthenes in one case and acetone in the other were detected by the relatively large residuals obtained when the analysis was first made, assuming that these constituents were not in the mixture.

The method of computation illustrated in Table I was described to show how the mass spectra of the components of a mixture superimpose to give the spectrum of the mixture and how the unused peaks may be used to check accuracy of the analysis. The particular method used for determining the separate components is applicable to only the simplest of analyses. A large amount of work has been directed to the development of short-cut methods for analyzing more complicated mixtures. The computing manual which explains these methods is over 100 pages in length.

Times Required for Analyses

The length of time required to make analyses with the mass spectrometer is very short, as is shown in the examples which follow.

Times are shown for the complete direct method of analysis. These times in most cases are somewhat longer than would normally be encountered in production analyses, as will be seen from the explanation below. In three examples (Tables IV, V, VI) times are also shown for the comparison method of analysis, which is faster and may be used for control purposes.

In the complete direct method calibrations of critical components of a mixture are run on the same day on which the mixture is run, and computations are carried through at a large number of masses in order to obtain a check on the accuracy of the analysis as well as to discover unexpected constituents. In general, calibrations will consume about 2 or 3 hours per day of mass spectrometer time when the direct method is used. In the examples which follow, the value given for man-hours includes a prorated time for the calibrations.

If it is not desired to check for unexpected compounds and general accuracy of analyses, it is unnecessary to carry through computations for a large number of masses. Considerable time can therefore be saved in computing and tak-

Table IV. Synthetic C_1 to C_4 Paraffin-Olefin Mixture

(Composition computed from the manometer synthesis is compared with mass spectrometer analysis.)

	Manometer Synthesis	Mass Spectrometer	Difference
	Mole per cent		
Methane	11.3	10.9	−0.4
Ethylene	1.5	1.7	+0.2
Ethane	22.1	21.4	−0.7
Propene	11.9	11.8	−0.1
Propane	31.1	31.0	−0.1
Isobutane	5.0	5.0	0.0
Isobutene	4.4	4.7	+0.3
Butene-1	5.0	6.2	+1.2
Butene-2	0.7	0	−0.7
n-Butane	7.0	7.3	+0.3

	Time Required for Analysis: Complete direct method *Min.*	Comparison (control) method *Min.*
Mass spectrometer (instrument time)		
Mixture	20	20
Prorated calibration	12	4
Taking data from record (technician's time)		
Mixture	20	15
Prorated calibration	12	1
Computing	130	80
Total man-hours	3.25	2
For *n*-, isobutane, and total butenes only, hour	0.75	..

Table V. Typical Feed Stocks

	Catalytic Polymerization			Acid Alkylation		
	Manometer	Mass spectrometer	Difference	Manometer	Mass spectrometer	Difference
	Mole per cent			Mole per cent		
Propene	1.0	1.1	+0.1	1.0	1.2	+0.2
Propane	2.1	2.0	−0.1	2.0	2.0	0
Isobutane	9.8	9.2	−0.6	14.8	14.9	+0.1
Isobutene	13.7	12.7	−1.0	0	0	0
Butene-1	15.0	16.5	+1.5	10.0	10.6	+0.6
Butene-2	14.0	14.1	+0.1	5.0	4.6	−0.4
n-Butane	42.4	42.4	0	64.2	63.8	−0.4
Isopentane	0.5	0.5	0	1.0	0.9	−0.1
n-Pentane	0.5	0.6	+0.1	0.5	0.5	0
Pentenes	1.0	0.9	−0.1	1.5	1.5	0

	Time Required for Analysis	
	Complete direct method	Comparison (control) method
	Min.	*Min.*
Mass spectrometer (instrument time)		
Mixture	25	25
Prorated calibration	10	3.5
Taking data from record (technician's time)		
Mixture	25	15
Prorated calibration	10	1.5
Computing	140	75
Total man-hours	3.5	2

ing data from the records in the partial direct method, since only the masses used directly in the solution need be considered. In some cases both the computing time and record reading time will be cut approximately in half.

The values given for total man-hours in the complete direct method should therefore be considered as upper limits. These times would normally be encountered only when using the mass spectrometer for experimental rather than production analyses.

If it is desired to use the spectrometer for control purposes, the time for analysis can be reduced below that attainable by either direct method, since in general there will be a large number of samples of similar composition. This permits the use of the comparison method of analysis, which eliminates a large portion of both calibration and computation and is therefore faster than the direct method.

The comparison method is essentially a comparison between an unknown sample and a standard sample. If the mass spectrometer is to be used for control purposes—for example, to indicate a change in the composition of a stream—very rapid results can be obtained with the comparison method. Such control can be accomplished merely by making periodic runs on the stream and comparing the mass spectra thus obtained with the mass spectrum of a standard mixture which is run once each day. Any change in the difference between the spectrum of the stream and the spectrum of the standard mixture will indicate a change in the composition of the stream. In addition, the change in composition can be computed from this difference spectrum; or a complete analysis can be made from this difference spectrum and a knowledge of the composition of the standard mixture.

The comparison method of analysis just described may be used to advantage whenever a large number of samples with similar composition are to be run in a single day. There are three main advantages in using this method: (1) Complete calibrations need be made only about once a month, thus reducing to practically zero the prorated calibration time. (2) All data necessary for complete analysis are obtained, but it is unnecessary to carry out computations unless the composition of the sample has changed a sufficient amount to be of interest. This reduces to zero the computing time for some samples. (3) When it is desired to obtain a complete analysis, the computing time is materially less than required by the direct method. The times given for the comparison method in Tables IV, V, and VI demonstrate the rapidity with which complete analyses can be obtained by this method.

With either the direct or comparison methods a large number of samples can be run in a day. For example, with a C_4 feed stream as illustrated in Table V, fourteen samples can be run by the direct method and seventeen samples by the comparison method in an 8-hour day. The corresponding numbers for the C_4 cut free of C_5 shown in Table VI are 16 and 24 samples per 8-hour day.

In some cases when only the abundance of one or two components of a mixture is required, the analysis can be made rapidly, whether or not there are a large number of samples of similar composition. For example, in a C_1 through C_4 paraffin-olefin mixture, if only the mole per cents of *n*- and isobutane and total butenes are desired, the total time is about 40 minutes. In the determination of butadiene in a C_1 to C_4 mixture or in a C_4 cut, the time is only 30 minutes.

Analyses Made with the Mass Spectrometer

Some important mixtures which have been analyzed with the mass spectrometer to date are:

1. Dry gas
2. Wet gas
3. *n*- and isobutane mixtures
4. C_1 through C_4 paraffin and olefin
5. C_3 through C_5 paraffin and olefin
6. C_4 paraffins, olefins
7. C_4 paraffins, olefins, and diolefin
8. C_5 paraffins, olefins, diolefins, and cyclic
9. C_5 through C_6 paraffins, cyclics, and aromatics
10. Determination of benzene, toluene, and xylenes in gasoline
11. Determination of small amounts of diethylbenzene in ethylbenzene

Examples of depropanizer overhead and *n*- and isobutane mixtures have been previously published (*1*).

Table VI. Synthetic C_4 Paraffin-Olefin-Diolefin Mixtures

	Mixture 1			Mixture 2			Mixture 3		
	Manometer	Mass spectrometer	Difference	Manometer	Mass spectrometer	Difference	Manometer	Mass spectrometer	Difference
	Mole per cent			Mole per cent			Mole per cent		
Propene	0.9	0.4	−0.5	0	0	0	0	0	0
Butadiene	66.6	67.2	+0.6	92.6	92.5	−0.1	3.8	3.8	0
Isobutane	0	0.6	+0.6	0	0	0	0	0	0
Isobutene	0.9	1.0	+0.1	0	0.2	+0.2	30.4	28.9	−1.5
Butene-1	7.1	8.1	+1.0	1.9	2.0	+0.1	31.4	29.2	−2.2
Butene-2	6.9	5.5	−1.4	5.5	5.3	−0.2	34.4	38.1	+3.7
n-Butane	17.6	17.2	−0.4	0	0	0	0	0	..

	Time Required for Analysis	
	Complete direct method	Comparison (control) method
	Min.	*Min.*
Mass spectrometer (instrument time)		
Mixture	20	15
Prorated calibration	7	5
Taking data from record (technician's time)		
Mixture	20	15
Prorated calibration	7	5
Computing	95	50
Total man-hours	2.5	1.5
Butadiene only, min.	35	..

Table VII. C_5 Paraffin-Olefin-Isoprene-Cyclopentane Mixture

	Synthetic	Mass Spectrometer 5-6-42	5-7-42	5-8-42
	Mole per cent			
Isopentane	11.3	10.2	10.4	10.5
Pentene-1	28.8	31.4	25.7	31.6
Isoprene	19.0	19.7	20.3	20.2
n-Pentane	11.9	10.5	11.0	10.4
2-Methylbutene-2	19.3	19.0	20.6	18.7
Cyclopentane	9.7	9.2	12.1	8.6

	Time Required for Analysis by Complete Direct Method *Min.*
Mass spectrometer (instrument time)	
Mixture	25
Prorated calibration	28
Taking data from record (technician's time)	
Mixture	25
Prorated calibration	28
Computing	195
Total man-hours	5

Wet Gas Mixture. Table II shows the comparison between the results obtained on a wet gas mixture by a fractionating column and the mass spectrometer. The main point of interest in this analysis is that the mass spectrometer was able to detect small quantities of naphthenes.

Table III shows the comparison between two fractionating columns and the mass spectrometer. Perhaps the most interesting point in this example is the fact that the mass spectrometer was able to determine the presence of a small amount of acetone. The presence of acetone was probably caused by a contaminated sample bottle. If the mass spectrometer analysis is corrected for the presence of acetone, the maximum discrepancy between these corrected results and the fractionating column results is only 0.4 mole per cent.

These two examples illustrate the power of the mass spectrometer method to detect unexpected substances in a sample, even when these substances have the same molecular weight as other components in the mixture—the molecular weight of acetone is the same as that of *n*- and isobutanes.

C_1 to C_4 Paraffin-Olefin Mixture. Table IV shows the results of the analysis of a synthetic mixture containing C_1 to C_4 paraffins and olefins. In the column labeled "Manometer" is shown the composition of the mixture in mole per cent as synthesized with the aid of a mercury manometer. The next column shows the results of the analysis obtained with the mass spectrometer, while the last column shows the discrepancy between the manometer and mass spectrometer determinations.

The example in Table IV was selected to illustrate what is probably the authors' average accuracy on this type of mixture. In general, the total butenes and separate paraffins are determined to within the same accuracy. However, the separation of the butenes, and particularly the separation of butene-1 and butene-2, is less accurate because of the similarity of their mass spectra. Average errors in determining the separate butenes are about three times the average errors in determining the separate paraffins.

If small amounts of C_5 (say 1 per cent) are present in a mixture such as shown, the times are increased by about 15 minutes. The probable error in separating the butenes is increased about 0.5 per cent.

If larger amounts of C_5 paraffins and olefins are present, the accuracy of the determination of the total butenes is not affected. The determination of the accuracy with which the butenes can be separated in the presence of large amounts of C_5 is at present awaiting additional data.

C_3 to C_5 Paraffin-Olefin Mixture. Table V shows the results obtained on two synthetic mixtures with compositions similar, respectively, to polymerization plant and alkylation plant feed streams. These mixtures contain a small amount of C_5 paraffins and olefins. The results show that good accuracy can be obtained on the separate determination of the C_4's, when as much as 3 per cent of the C_5's is present. The effect on accuracy and analysis time of the presence of larger amounts of pentenes is at present awaiting additional data, as in the previous example.

C_4 Paraffin-Olefin-Diolefin Mixture. Table VI gives analyses of three synthetic C_4 mixtures containing butadiene in addition to paraffins and olefins. In each case the composition as obtained in the manometer synthesis is compared with the mass spectrometer analysis. The presence of small or large amounts of butadiene in a mixture of this type can be detected with an accuracy of about ±0.3 per cent. When the partial concentration of butadiene is about 50 per cent, the average accuracy of its determination is about ±0.7 per cent. The accuracy of determining the other components of these mixtures is only slightly affected by the presence of butadiene.

The third analysis shows a relatively large error in the separation of butene-1 and butene-2. In spite of the fact that large amounts of butenes are present, this error is larger than that normally encountered. It is included here, so that the poor as well as the typically good results will be illustrated.

C_5 Paraffin-Olefin-Diolefin and Cyclic Mixture. Table VII compares three mass spectrometer analyses obtained on three consecutive days on a synthetic C_5 mixture containing paraffins, olefins, isoprene, and cyclopentane, with the composition reported from the synthesis. This mixture did not contain all the C_5 olefins or diolefins, simply because the pure components were not available. However, the mixture could still be analyzed if all components normally encountered in such a mixture were present, although the accuracy in separating the individual olefins would probably not be so good as is shown in this table. "Synthetic" rather than "Manometer" is used in the tables whenever the sample was prepared by a laboratory other than the Consolidated Engineering Corporation.

Table VIII. C_6 Paraffin Synthetics

(Two C_6 mixtures were analyzed by the mass spectrometer method on two successive days. Composition from synthesis is given for comparison.)

	Synthetic	Mass Spectrometer 6-25	6-26	Synthetic	Mass Spectrometer 6-25	6-26
	Mole per cent			*Mole per cent*		
2,2-Dimethylbutane	3.82	3.5	3.5	26.40	26.1	25.9
Cyclopentane	0	0	0	0	0	0
2,3-Dimethylbutane	0	0	0.3	0	0.5	0.2
2-Methylpentane	12.03	12.5	12.3	36.24	35.7	36.4
3-Methylpentane	6.16	6.8	7.3	20.26	20.8	20.6
n-Hexane	77.99	77.2	76.6	17.10	16.9	16.9

	Time Required for Analysis by Complete Direct Method *Min.*
Mass spectrometer (instrument time)	
Mixture	25
Prorated calibration	12
Taking data from record (technician's time)	
Mixture	25
Prorated calibration	12
Computing	120
Total man-hours	3.25

C_6 Paraffin Mixture. Table VIII shows two analyses of synthetic mixtures containing C_6 paraffins. In each case, the analysis was made on two different days to see how well the mass spectrometer results would reproduce on this type of mixture.

C_5-C_6 Paraffin-Cyclic and Aromatics. Table IX shows the analysis of a mixture containing C_5 and C_6 paraffins, cyclics, and aromatics. Since this mixture was not a syn-

Table IX. Mixture of C_5 and C_6 Paraffins, Cyclics, and Aromatics

Hydrocarbon	Fractionation (100-Plate) Column	Ultraviolet Absorption	Mass Spectrometer	Difference	
	Mole per cent				
Pentanes	3.8	..	3.7	−0.1	
2,2-Dimethylbutane	14.0	..	15.0	+1.0	
Cyclopentane	1.6	..	2.0	+0.4	
2,3-Dimethylbutane	9.4	..	7.7	−1.7	
2-Methylpentane	33.0	..	32.2	−0.8	
3-Methylpentane	13.9	..	14.6	+0.7	
n-Hexane	12.8	..	13.5	+0.7	
Methylcyclopentane	8.7	..	9.3	+0.6	
Benzene	2.9	2.0	2.1	−0.8	+0.1

	Time Required for Analysis by Complete Direct Method
	Min.
Mass spectrometer (instrument time)	
Mixture	25
Prorated calibration	23
Taking data from record (technician's time)	
Mixture	25
Prorated calibration	23
Computing	160
Total man-hours	4.25

thetic, the table shows the comparison between the results obtained with a mass spectrometer and by a combination of refractive index readings and temperature readings taken on a large number of small fractions obtained from a 100-plate fractionating column. The two analyses are in fairly good agreement. Results of the determination of benzene by ultraviolet absorption are also shown.

The time required by the mass spectrometer in this case is extremely short as compared with the fractionating time which in this case was 10 days, 24 hours a day. Even in the case where the unknown mixture contains C_7's in addition to C_5 and C_6, a preliminary relatively rapid fractionation can be made to cut out the C_7's, and the total time for preliminary fractionation and the mass spectrometer analysis is still materially shorter than the time required by fractionation alone.

Gasoline and Aromatics. Table X shows the analysis for the benzene, toluene, and xylene in a synthetic gasoline. If ethylbenzene had also been present in this mixture, its amount could have been separately determined. The major portion of the analysis time given was consumed in preparation of the sample preliminary to its introduction into the mass spectrometer. This preparation is done on an auxiliary apparatus and so does not consume any spectrometer time. The time for the actual spectrometer run and prorated calibration is only 28 minutes. Combined computing and instrument time is thus only 45 minutes. Sample preparation time for this type of mixture can be materially reduced as the bottling method is improved.

Determination of Diethylbenzene in Ethylbenzene. Table XI shows the results of eight tests on the determination of small quantities of diethylbenzene in ethylbenzene. This example is representative of a number of analyses which can be made with the mass spectrometer to check the purity of a particular compound. In this case the minimum amount of impurity that could be detected without making special adjustments is about 0.02 per cent. If a small amount (down to 0.02 per cent) of isopropylbenzene were also present in these mixtures, its quantity could be determined.

Mass Spectrometer for Commercial Use

In order to make the mass spectrometer useful for routine analysis it was necessary to develop an instrument which was reliable, reasonably simple to operate, and at the same time capable of obtaining accurate mass spectra of gas and liquid mixtures.

The accuracy of the Consolidated Engineering Corporation mass spectrometer is demonstrated by the results of the analyses given above.

The commercial apparatus has been designed conservatively, so that it will require a minimum of service. In order to test the reliability of operation, the mass spectrometer in this laboratory was operated in a routine manner over a period of 4 months, during which time from 15 to 20 runs a day were made. This number of runs was obtained in an 8-hour day. However, in order to simulate 24-hour-a-day operation, all voltages were left on at all times during the 4 months with the exception of Sundays. During this period, in addition to many experimental runs, over 200 analyses were carried out on synthetic mixtures made in the laboratory and on mixtures sent in by oil companies which were interested in the possibilities of this new method of analysis.

Figure 3 shows a commercial model of the Consolidated Engineering Corporation mass spectrometer. In the center background is the electromagnet which produces the uniform magnetic field in which the mass spectrometer proper is placed. Immediately to the left of this magnet is the sample inlet system and evacuation apparatus. In the right foreground are the oper-

Table X. Synthetic Gasoline and Aromatics

(Composition obtained from the synthesis is compared with the mass spectrometer analysis for two mixtures containing aromatics and a large number of other hydrocarbons.)

	Synthetic	Mass Spectrometer	Difference	Synthetic	Mass Spectrometer	Difference
	Mole per cent			Mole per cent		
Nonaromatics[a]	49.1	49.1	0	52.9	55.0	+2.1
Benzene	12.7	12.5	−0.2	5.7	5.7	0
Toluene	37.9	38.4	+0.5	21.0	21.8	+0.8
Xylene	..	..	..	20.3	17.5	−2.8

	Time Required for Analysis by Partial Direct Method
	Min.
Mass spectrometer (instrument time)	
Mixture	25
Prorated calibration	3
Taking data from record (technician's time)	
Mixture	10
Prorated calibration	1
Computing	10
Sample preparation	120
Total man-hours	2 hours 50 minutes

[a] Nonaromatics: *n*-hexane, 2-methylpentane, 2,3-dimethylbutane, methylcyclopentane, cyclohexane, *n*-heptane, methylcyclohexane, "isooctane", and "isooctene".

Table XI. Small Amounts of Diethylbenzene in Nearly Pure Ethylbenzene

(Determination of diethylbenzene in ethylbenzene in eight different concentrations. Impurity added in synthesis is compared to mass spectrometer analysis.)

Mixture	Synthetic	Mass Spectrometer	Difference
	Mole per cent		
1	0.036	0.033	−0.003
2	0.071	0.066	−0.005
3	0.235	0.22	−0.02
4	0.479	0.44	−0.04
5	0.789	0.76	−0.03
6	2.41	2.2	−0.2
7	4.02	3.6	−0.4
8	7.97	8.0	0

	Time Required for Analysis by Partial Direct Method
	Min.
Mass spectrometer (instrument time)	
Mixture	25
Prorated calibration	3
Taking data from record (technician's time)	
Mixture	10
Prorated calibration	1
Computing	10
Sample preparation	120
Total man-hours	2 hours 50 minutes

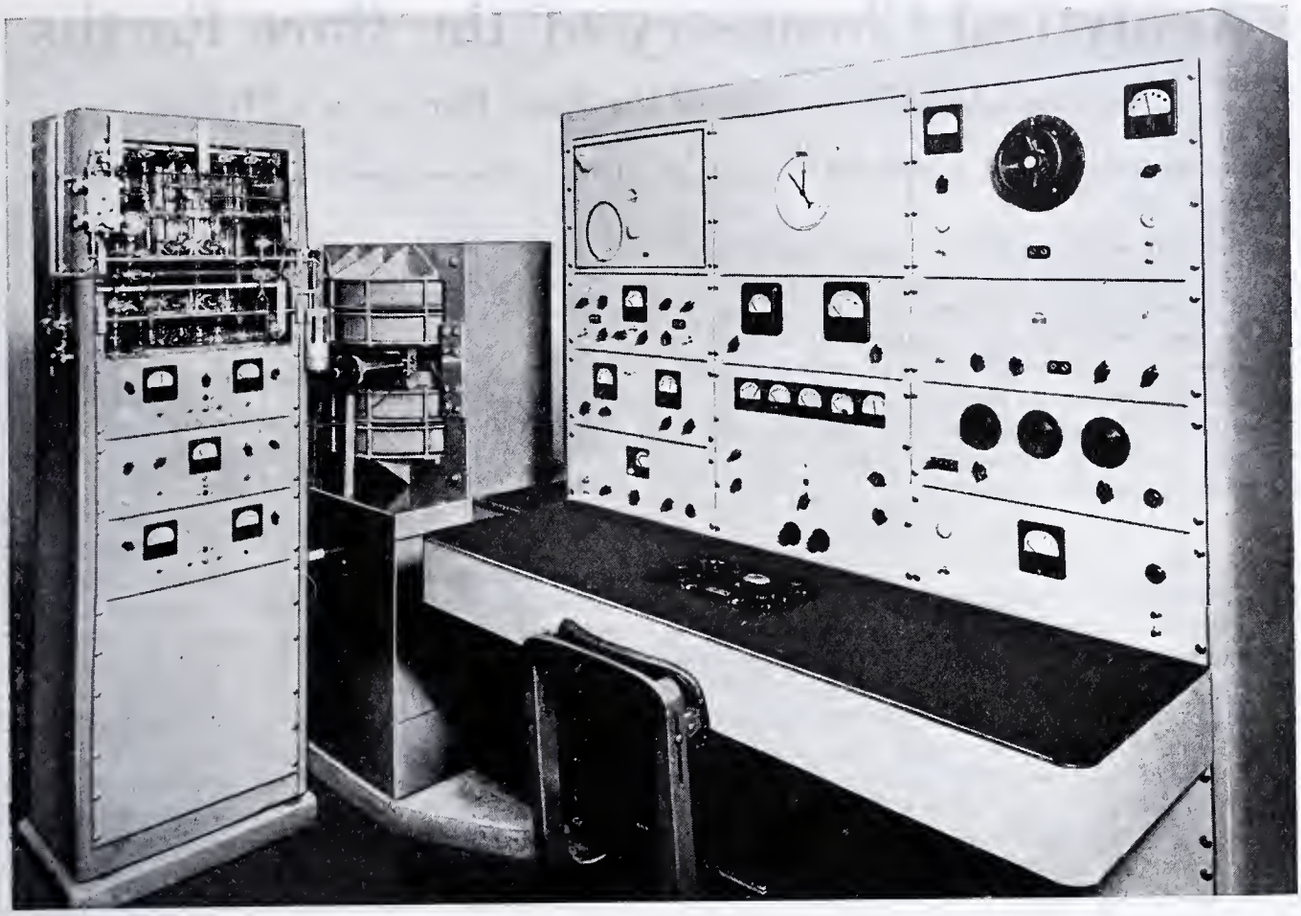

FIGURE 3. COMMERICAL MODEL OF THE CONSOLIDATED MASS SPECTROMETER

ator's table and the cabinet which houses the automatic recording apparatus and power packs. These power packs supply closely regulated voltages to the spectrometer and electric power to the magnet.

The routine operation of the apparatus for analyzing gas and liquid mixtures is relatively simple. The main operations are (1), the manipulating of the proper stopcocks to introduce the sample into the apparatus, and (2), the throwing of the proper switches to initiate the automatic recording of the data.

The data are recorded on photographic paper by a recording oscillograph.

In order to increase the reliability of the instrument and further to simplify its operation, several automatic protective devices have been installed to avoid damage to the instrument in an emergency. To a large extent, these protective features prevent the loss of operating time and allow relatively unskilled operators to take data.

The handling of sampling and sample containers in a refinery may be greatly simplified by using the mass spectrometer method, since the sample required is so minute. Only 0.1 cc. of gas at atmospheric pressure need be introduced into the inlet sample bottle. This extremely small size may be of decided advantage in some laboratory experiments.

In the refinery the size of sample should be picked which is best adapted to easy sampling and handling. Cylinders of about 100-cc. capacity should be very satisfactory.

Summary

The mass spectrometer method has several distinctive features:

1. A large number of samples, in some cases as many as 20, can be analyzed in an 8-hour day with one mass spectrometer
2. Mixtures containing up to 15 or more components can be analyzed.
3. Results can be computed in such a manner as to indicate whether the analysis is sufficiently accurate, and whether any unexpected components are present.
4. Results are practically independent of the skill and judgment of operators and computers.
5. Only a small sample is required for analysis, 0.1 cc. usually being sufficient.

Conclusion

As indicated by the examples given, advances in techniques of analysis using the mass spectrometer have been extremely rapid. As in the case of any new development, the field is constantly expanding. New problems extending the use of the instrument are continually being proposed and solved. Evidence is accumulating which indicates that analyses which are prohibitively time-consuming by other methods will be relatively readily analyzed by means of the mass spectrometer. The examples given in this paper should therefore not be considered as representing the limits of the method.

Acknowledgment

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Analytical Chemistry of the Rare Earths

Average Atomic Weight of the Rare Earths in a Mixture

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The oxalate to oxide, oxalate to permanganate, method for determination of the average atomic weight of the rare earth in a mixture has been studied, using pure materials and mixtures of known composition. An important source of error lies in the standardization of the permanganate solution. To minimize the error this operation should employ as a standard that rare earth which is the principal component of the mixture.

A KNOWLEDGE of the average atomic weight of the rare earths in a mixture allows the composition to be calculated if only two earths are present or provides sufficient data for such a calculation if the percentages of all but two of the components may be determined in other ways.

Analytical methods suitable for routine determinations of the atomic weights of the rare earths are few. The oxide to sulfate method of Schützenberger (*7*) is of historical interest only, since the many possible errors in the conversion to anhydrous sulfate render its use unreliable. The choice is apparently among the method of Feit and Przibylla (*3*) in which a weighed amount of oxide is dissolved in excess dilute sulfuric acid and the excess titrated with standard base; the oxalate to oxide, oxalate to permanganate, method of Gibbs (*5*); and the chloride to silver method, first used for a rare earth by Baxter and Chapin (*1*). Of these the first-named is unreliable for mixtures of the earths of weaker basicity, while the last is too time-consuming for routine application. The oxalate method is probably intermediate with regard to both the time required for the determination and the accuracy obtained.

The method involves the following steps: precipitation, washing, and oven-drying of the oxalate; determination of the oxalate to oxide ratio by the ignition of weighed portions of the partially dehydrated oxalate, followed by dissolution of weighed portions of the same sample of oxalate in sulfuric acid and titration of the oxalate content with standard permanganate solution. The average atomic weight is calculated from the formula, $A/B = (2\,R.E. + 3O)/3C_2O_3$, where A is the weight of rare earth oxide, $R.E._2O_3$ which corresponds to B, the weight of C_2O_3 found in the titrated samples.

Experimental

A preliminary investigation of the accuracy of the method was made, using certain spectrographically pure rare earths of the cerium group. The data are shown in Table I. The calculation:

$$\frac{2La + 3O}{3C_2O_3} = \frac{\dfrac{\text{Gram of oxide obtained}}{\text{Gram of oxal. ignited}} \times \text{gram of oxalate titrated}}{N \text{ of } KMnO_4 \times \text{m. eq. of } C_2O_3 \times \text{ml. of } KMnO_4 \text{ used}}$$

$$\frac{2La + 48}{216.06} = \frac{\dfrac{0.1863}{0.3208} \times 0.1218}{\dfrac{0.02526 \times 72.02 \times 51.40}{2 \times 1000}}$$

$$La = 139.45$$

While the precision of the experimental results is satisfactory for the desired purpose, the accuracy is not. In all cases the atomic weights are slightly high.

As a result of searching the method for errors leading to high values it appeared likely that the oxalate titer of the permanganate against National Bureau of Standards sodium oxalate (dried overnight at 140° C.) was not the same as that obtained against the oxalate content of rare earth oxalate dried at 110° C. for a similar period of time. A possible explanation of this, in turn, might be that some nitrato-oxalate (*6*) contaminates the oxalate of the rare earths when the latter is precipitated in the usual way (*4*).

A comparison was therefore made between the normality of potassium permanganate solutions as determined by standardization against Bureau of Standards sodium oxalate on the one hand and against the pure rare earth oxalate itself on the other. Table II lists the results obtained in this series of determinations. It is evident that the oxalate titer of the permanganate solution as determined against the oxalate of the rare earth concerned is greater than that determined against sodium oxalate except in the case of gadolinium. The trend is consistent and is toward closer agreement of the two normalities with decrease in basicity of the rare earth (increase in atomic number) and the change in this difference may be significant.

TABLE I. ATOMIC WEIGHTS OF SOME CERIUM GROUP EARTHS

Rare Earth Oxide	Weight of Oxalate[a] Titrated	Volume of $KMnO_4$ Used	N $KMnO_4$ *vs.* $Na_2C_2O_4$	Weight of Oxalate Ignited	Weight of Oxide Obtained	Calcd. Atomic Weight	International Atomic Weight, 1941
	Gram	*Ml.*		*Gram*	*Gram*		
La_2O_3	0.1218	51.40	0.02526	0.3208	0.1863	139.45	
	0.1090	45.80	0.02526	0.3953	0.2294	140.03	
					Av.	139.74	138.92
Nd_2O_3	0.1022	40.50	0.02515	0.2214	0.1247	145.54	
	0.0860	34.12	0.02515	0.2338	0.1321	145.88	
					Av.	145.71	144.27
Sm_2O_3	0.1132	43.15	0.02467	0.2099	0.1153	151.25	
	0.0896	34.10	0.02467	0.3056	0.1673	150.93	
					Av.	151.09	150.43

[a] It follows from the data in Table I that the weight of water remaining in the oxalate after partial dehydration at 110° C. could be calculated readily. Such materials are no longer hydrated with 9 or 10 molecules of water although they may have been precipitated as ennea- or decahydrates. Further investigation of this point is planned.

In view of these results it was decided that the permanganate solution in all cases should be standardized against a pure sample of the oxalate of that rare earth which appears to be most abundant in the mixture concerned.

Recommended Procedure

The experimental procedure is the same for the standardization of the permanganate solution against the oxalate of a rare earth of known atomic weight as it is for the use of the permanganate thus standardized in the determination of the

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Table II. Comparison of Standardization Methods

Rare Earth Oxide	Weight of Oxalate Titrated	Volume of $KMnO_4$ Used	Weight of Oxalate Ignited	Weight of Oxide Obtained	N $KMnO_4$ vs. R. E. Oxalate	N $KMnO_4$ vs. $Na_2C_2O_4$
	Gram	*Ml.*	*Gram*	*Gram*		
La_2O_3	0.1195	51.20	0.2005	0.1178	0.02525	0.02494
	0.0782	33.55	0.2017	0.1183	0.02517	0.02494
	0.0982	41.00	0.4747	0.2761	0.02565	0.02545
	0.1303	54.50	0.6367	0.3703	0.02561	0.02545
Nd_2O_3	0.1421	55.35	0.1410	0.0775	0.02516	0.02494
	0.1275	49.60	0.1912	0.1049	0.02514	0.02494
	0.1301	51.70	0.5301	0.3034	0.02568	0.02545
	0.1562	62.00	0.4457	0.2551	0.02571	0.02545
Sm_2O_3	0.1595	60.70	0.2544	0.1410	0.02505	0.02494
	0.1251	47.65	0.2530	0.1405	0.02508	0.02494
	0.2386	89.40	0.4198	0.2347	0.02566	0.02545
	0.1043	39.20	0.6214	0.3468	0.02554	0.02545
Gd_2O_3	0.1528	56.95	0.5778	0.3238	0.02488	0.02494
	0.1182	44.10	0.8658	0.4851	0.02485	0.02494
	0.1784	64.60	0.5724	0.3205	0.02559	0.02567
	0.1487	53.85	0.4277	0.2394	0.02557	0.02567

Table III. Standardization against Lanthanum Oxalate

Sample	Weight of Oxalate Titrated	Volume of $KMnO_4$ Used	Weight of Oxalate Ignited	Weight of Oxide Obtained	Oxide Equivalent to Oxalate Titrated	M. Eq. of C_2O_3	N of $KMnO_4$
	Gram	*Ml.*	*Gram*	*Gram*	*Gram*		
I	0.1792	47.80	0.2005	0.1178	0.1053	0.03601	0.04056
II	0.1564	41.70	0.2017	0.1183	0.0917	0.03601	0.04052

average atomic weight of an unknown mixture. The calculations involving data from the former operation are shown below.

About 1 gram of the pure rare earth oxide standard (or of the unknown oxide mixture) is dissolved in 50 ml. of water containing 7 ml. of 15 N nitric acid. After complete dissolution has occurred, the solution is diluted to 100 ml. and heated to boiling and the rare earth oxalate is precipitated by the slow addition of a hot solution of 10 grams of oxalic acid dihydrate in 50 ml. of water.

Since the presence of even small amounts of oxalic acid in the precipitate will seriously affect the final result, it is important that the oxalate be thoroughly washed. This operation should consist of two washings by decantation with 100-ml. portions of hot water followed by a washing on a filter, removal to a beaker, and another series of decantations. After a final washing on a filter the wet oxalate is removed to a beaker, the supernatant liquid decanted, and the precipitate dried at 110° C. for 8 to 12 hours.

From the partially dehydrated oxalate are removed two samples, each of about 0.2 gram, and these are accurately weighed and ignited in an electrically heated muffle furnace to constant weight at 900° C. In this ignition it is advisable to put the oxalate into a cold furnace and to allow the temperature to rise slowly, since the dry powder is very easily blown from the crucible by the escaping gases if rapid heating next to the walls is allowed to occur.

Two other oxalate samples, each of about 0.15 gram, are removed, accurately weighed, and dissolved in 20 ml. of warm 10 N sulfuric acid. These solutions are diluted with 100 ml. of water, heated to 90°, and titrated with standard potassium permanganate solution. The normality of this oxidant is conveniently either 0.04 or 0.025 N (*ca.* 1.26 or 0.79 gram of potassium permanganate per liter of solution), depending upon the amount of pure material available for standardization as well as upon the weight of sample available for the desired study.

The data in Table III illustrate the standardization of such a permanganate solution against lanthanum oxalate.

$$\frac{2La + 3O}{3C_2O_3} = \frac{0.1053}{x\,N\,KMnO_4 \times 47.80 \times 0.03601} = \frac{2 \times 138.92 + 3 \times 16}{3 \times 72.02}$$

$$x = 0.04056$$

Results and Discussion

In order to examine the usefulness of the method, a binary mixture of known composition was prepared and studied. Accurately weighed amounts of pure lanthanum oxide and samarium oxide were taken and dissolved in nitric acid, the solutions were combined, and the oxalates were precipitated together. The average atomic weight of the rare earth in the mixture was then determined according to the recommended procedure. The permanganate used was standardized against lanthanum oxalate, the principal constituent of the mixture, as well as against Bureau of Standards sodium oxalate, and both of the resulting normalities were used in the calculation. The results are shown in Table IV. It must be concluded that the accuracy obtained is much greater if standardization against the rare earth oxalate is employed.

The oxalates of cerium, praseodymium, terbium, and possibly of neodymium yield on ignition various oxides containing some or all of the rare earth in a higher oxidation state. Their exact behavior in mixtures is not yet certainly defined nor the influence of cerium certainly understood. It is therefore advisable to remove cerium before the atomic weight is determined; this may be accomplished quantitatively by the use of the iodate method (*4*). Praseodymium and terbium cannot be thus removed, so that their behavior on ignition must be accepted, for the present, as resulting in an unavoidable error. These elements are rare, however, and for usual mixtures the error will be small. For mixtures rich in these elements the ignition product may be assumed to be Pr_6O_{11} (*2*) and Tb_4O_7 (*8*). The behavior of neodymium under the prescribed ignition conditions is still incompletely known, but it is certain that it is not oxidized to a valence higher than 3 when cerium alone is present. The influence of cerium and praseodymium together has not yet been fully investigated, but the behavior of these elements in the ignition of various mixtures is being studied and will form the subject of a later paper in this series.

Table IV. Analysis of a Mixture of Known Composition

Standard	N $KMnO_4$	Atomic Weight of Mixture	La Calcd.	Sm Calcd.	Actual La	Actual Sm
			%	%	%	%
$Na_2C_2O_4$	0.02560	143.48[a]	60.4	39.6	69.2	30.8
$La_2(C_2O_4)_3$	0.02580	142.20[b]	71.5	28.5	69.2	30.8

[a] Average of 143.78 and 143.17.
[b] Average of 142.48 and 141.91.

Acknowledgment

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Potential Service Performance of Lubricating Oils

Laboratory Evaluation

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The corrosion and stability apparatus and method of test are introduced as means for the preliminary evaluation of the quality of lubricants. The distinctive feature of the test is the use of a felt wiping action on the bearing surface to simulate the action of the journal in the engine. Application of the test to twenty oils upon which extensive engine data have been reported establishes it as capable of predicting the oxidation stability and potential corrosivity of lubricants with accuracy sufficient to warrant the use of the test as a useful and reliable tool, in both research and control, to eliminate much of the more costly and time-consuming engine testing.

IT IS universally agreed that the ultimate criterion of the quality of a lubricant is efficient performance in the engine for which it is designed. This fact will remain permanent, since the development of bench methods, which seek optimum control of variables and elimination of extraneous factors, inevitably leads to departures from the actual engine. It is scarcely possible that accurate reproduction of an engine in its entire scope will ever be realized in a noncombustion type of laboratory apparatus.

The existence of many laboratory apparatus and methods of test, several of comparatively recent origin, testifies to the need for means of evaluating oils which can cope economically and practically with the great bulk of testing required either in the development of improved lubricants or in the control of oils in production. Such service is far too formidable for even small-scale test engines. When laboratory tests are available in such numbers, the presentation of a new apparatus perhaps requires justification. It is generally true that each test was developed specifically to evaluate a particular feature which the originators deemed important and worthy of emphasis. The tests, existent prior to the development of the present apparatus, represented a wealth of knowledge and experience from which could be selected the outstanding feature of individual cases, for incorporation into the new corrosion and stability apparatus. For example, severe conditions of aging lead to the simultaneous occurrence

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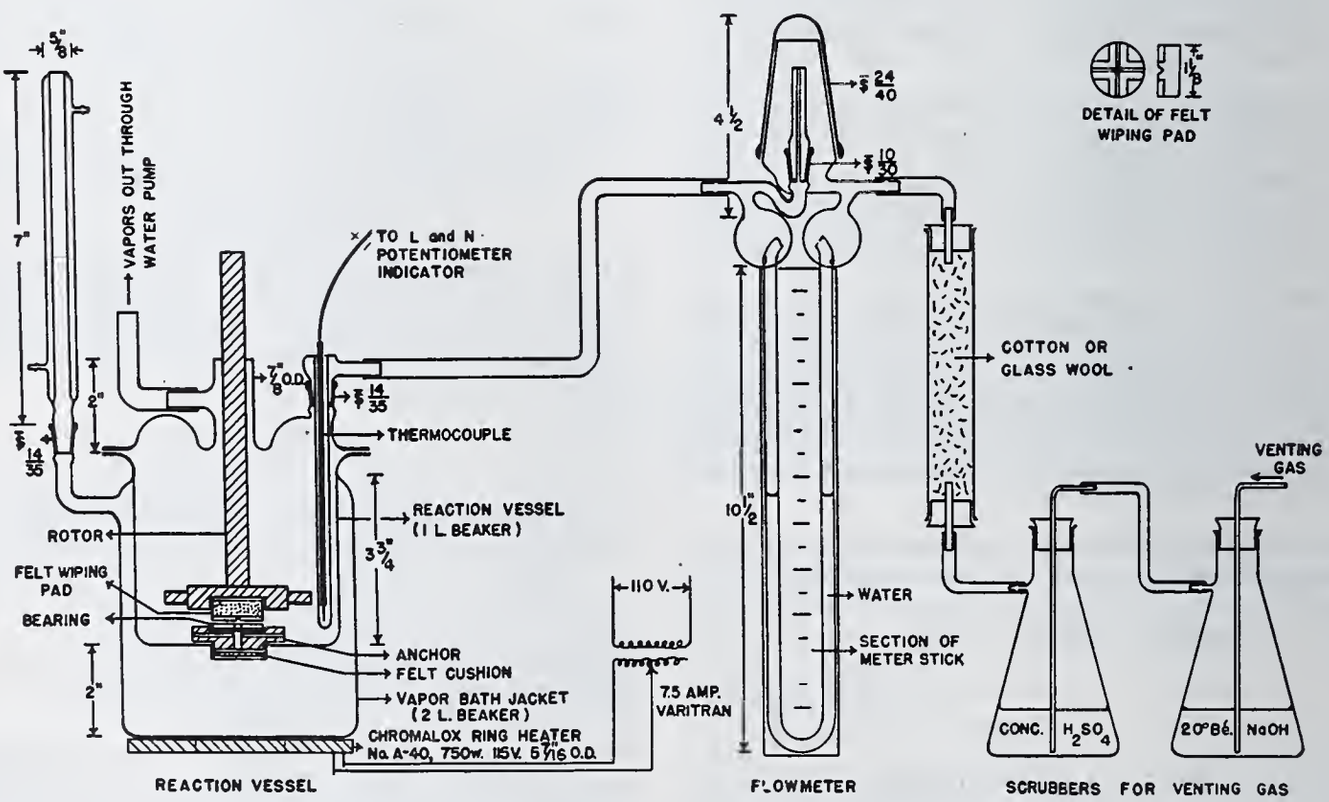

FIGURE 1. DETAIL AND FLOW DIAGRAM OF CORROSION AND STABILITY APPARATUS

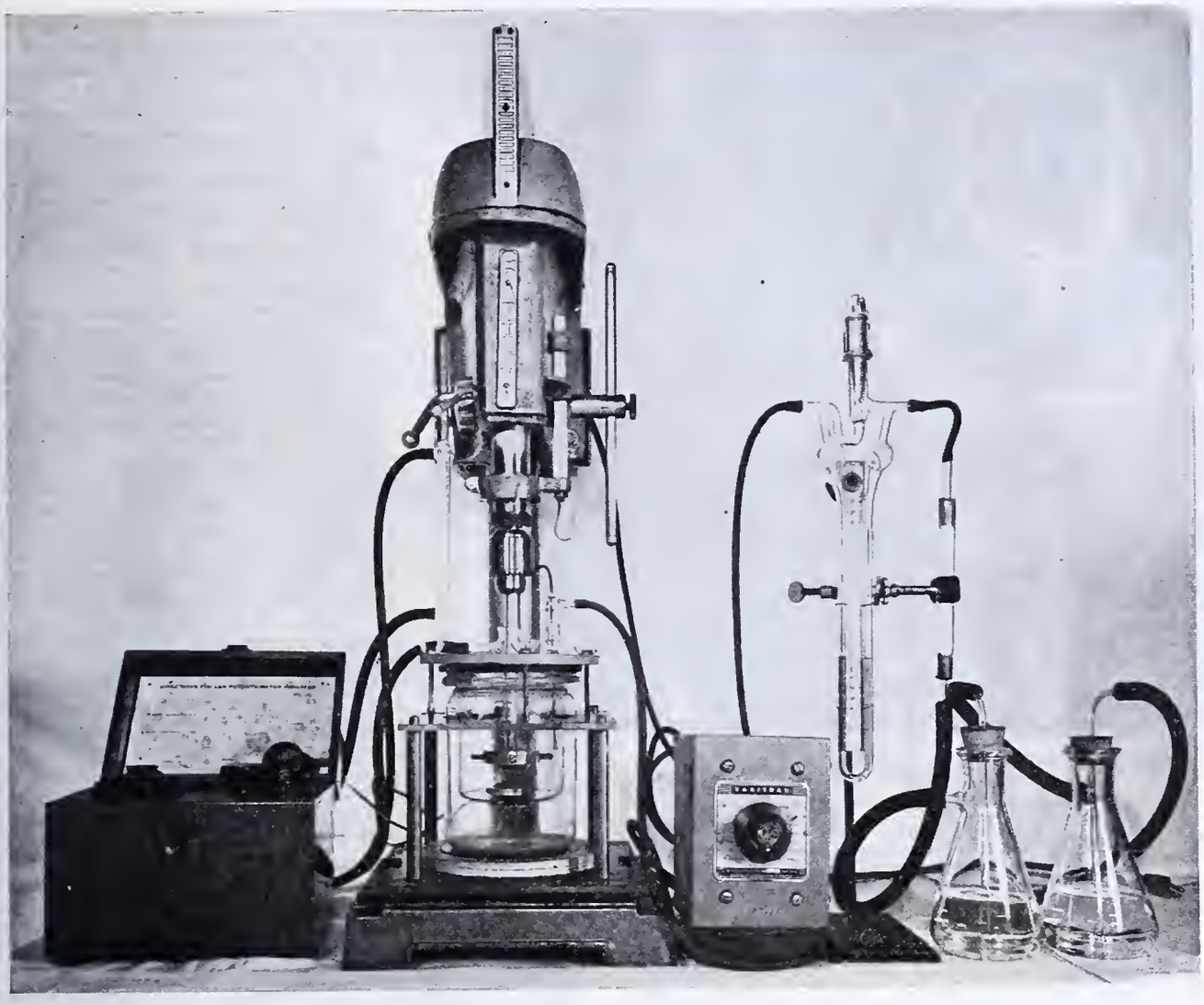

FIGURE 2. CORROSION AND STABILITY APPARATUS
Metal support for reaction chamber removed and rotor raised

of oil oxidation and corrosion, a feature of several tests; a large oil charge facilitates the determination of products of deterioration and evaluation of oxidation stability, an objective of the Underwood test (*11*); freedom from materials other than glass and aluminum eliminates stray catalytic effects and lessens the cleaning problem; the use of a vigorous stirring action in the oil acknowledges a merit common to the Indiana stirring oxidation (*5*) and the MacCoull corrosion (*6*) tests; venting the reaction chamber with air is a feature also common to many tests; finally, the use of a felt wiping action on the bearing surface, which resembles more closely the earlier technique of Clower (*3*) than that of the thrust bearing corrosion machine (*10*), incorporates a feature recently suggested as essential in the accurate evaluation of corrosivity of oils toward copper-lead bearings. In addition, the new apparatus and method of test have been designed to constitute a durable, inexpensive apparatus capable of evaluating in 5 hours the essential characteristics of a lubricant, and hence are amenable to either research or control application.

Corrosion and Stability Apparatus

A major premise upon which the present apparatus and test are based is that it is possible, empirically and artificially, to age an oil in the laboratory in a manner comparable with the aging which it experiences under conditions of service. Rigid control of the important variables, temperature, catalysis, time, and concentration of reactants, is important to obtain satisfactory reproducibility. Compatible with the above premise, simplicity in design has been sought rather than reproduction of any mechanical features of an engine. It is recognized, however, that factors, operative and influential in an engine, must of necessity remain extrinsic to a laboratory test—for example, fuel, fuel combustion products, dirt, etc. The correlation between laboratory test and engine is thereby limited, and the necessity of retaining the engine as a final criterion over all laboratory tests becomes apparent. The degree of correlation which has been obtained between engine performance and the corrosion and stability test is sufficient to establish the latter as a useful tool for the preliminary evaluation of lubricants.

APPARATUS. The corrosion and stability apparatus is illustrated in complete assembly by diagram in Figure 1 and by photograph in Figure 2. In Figure 3 the glass parts of the apparatus are pictured dismantled and in Figure 4 the metal and felt units are similarly illustrated.

For convenience in description, the apparatus can be divided into four assemblies.

Reaction Vessel (Figures 1 and 3). The reaction vessel is fashioned out of two Pyrex beakers of 1- and 2-liter capacity, which are joined at their rims by a ring seal. The space between the beakers, which is equipped with a reflux condenser, serves as a vapor bath and provides a convenient and precise method for supply of heat and control of temperature.

The heat medium may be any material which has a constant boiling point coincident with the desired temperature of test.

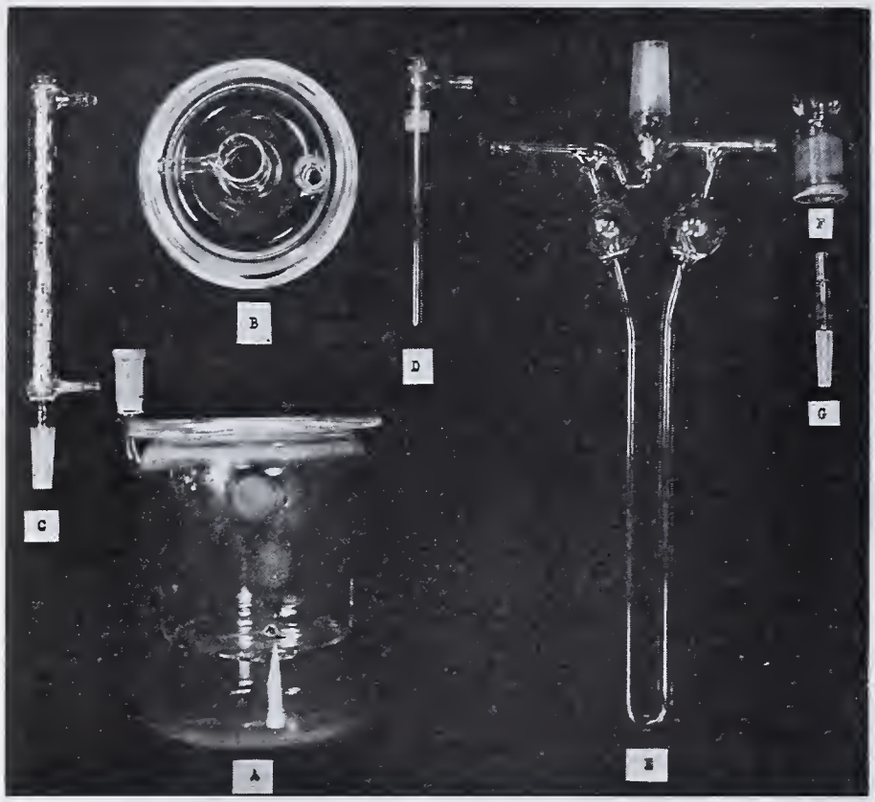

FIGURE 3. GLASS EQUIPMENT OF CORROSION AND STABILITY APPARATUS

A. Reaction vessel and vapor bath
B. Cover
C. Reflux condenser
D. Combined thermocouple well and air inlet
E, *F*, *G*. Manometric flowmeters

For standard testing, aniline (b. p. 363° F.), conventionally purified by drying and redistillation, is most frequently used. However, wide ranges of temperature have been covered in application of the apparatus by use of the following materials: water (212° F.), chlorobenzene (270° F.), bromobenzene (313° F.), pseudocumene (337° F.), aniline (363° F.), and naphthalene (424° F.). In the bottom of the inner beaker there is a square recess, slightly larger than 2.8 × 2.8 cm. (1.125 × 1.125 inches) and about 0.6 cm. (0.25 inch) in depth. The reaction chamber is covered by an essentially flat glass lid, and a seal is effected by a flat ground-glass contact between the lip of the reaction chamber and the cover. Through the center of the cover passes the shaft of the aluminum rotor. Also from the center of the cover extends a lead to a water aspirator which serves to prevent fumes of deteriorating oil from being dispersed into the laboratory. Displaced to one side of the central opening through the cover there is a female ground-glass joint which accommodates a combined thermocouple well and an air inlet to the reaction chamber. Through this opening, samples of oil can be taken, if desired, with only momentary interruption of operation. The over-all volume of the reaction chamber is approximately 850 to 900 ml.

Wiping and Stirring Assembly (Figures 1 and 4). Into the square recess in the bottom of the reaction chamber fits the aluminum bearing insert holder, called the "anchor" (Figure 4, *C*), which consists of an aluminum disk, from the bottom of which extends a square protrusion and into the top of which is sunk a square recess. The square protrusion from the bottom of the anchor fits into the recess in the bottom of the reaction chamber and is cushioned by a square felt pad cut to dimensions and about 0.3 cm. (0.125 inch) in thickness (Figure 4, *B*). This feature prevents rotation of the anchor during operation and holds the bearing insert stationary during a run. The recess in the top of the anchor accommodates the square bearing inserts, 2.8 × 2.8 cm. (1.120 × 1.120 inches), and of thickness 0.25 cm. (0.1 inch), sufficient for the surface of the bearing to extend about 0.75 cm. (0.03 inch) above the surface of the anchor (Figure 4, *D*).

Laterally through the cylindrical section of the anchor are bored four radial holes which are placed along two perpendicular diameters. These conduits converge in a larger hole bored coaxially through the anchor. A hole bored perpendicularly through the bearing insert is of the same size and coincides with the one extending coaxially through the anchor. This system of ducts through anchor and bearing insert provides a means which, in conjunction with the design of the felt wiping pad, ensures an even and continual flow of lubricant over the bearing surface. The bearing inserts, of course, can be of any available metal or alloy; copper, aluminum, lead, copper-lead, cadmium-nickel, and lead-flashed copper-lead have been employed.

The wiping and stirring actions are provided by an aluminum-felt unit which is called the "rotor" (Figure 4, *A*). The aluminum portion of the rotor consists of a shaft which is chucked into a conventional, bench-type drill press. At the lower extremity of the shaft is an aluminum disk, extending up into the bottom of which is a cylindrical recess placed coaxially with the disk and shaft. Extending down from the "ceiling" of the recess are aluminum spikes which embed themselves in the felt wiping pad (Figure 4, *E*) and prevent its slipping during rotation. The felt wiping pad is fashioned out of a cylindrical pad of felt of diameter such that a snug fit is ensured with the recess in the rotor and of sufficient thickness (0.9–1.25 cm., 0.375–0.5 inch) to extend about 0.3 cm. (0.125 inch) below the surface of the rotor. In general, the nature of the felt used is not critical, a limiting factor being that it shall not disintegrate during the course of a run. Normally, SAE F3 gray backcheck felt (95 to 100 per cent wool) is used; SAE F1 white backcheck (100 per cent wool) has been found satisfactory.

On the face of the pad, which wipes the bearing insert during operation, is cut a pattern which is represented in the diagram of Figure 1, and shown by photograph in Figure 4, *E*. Briefly, this pattern, which is cut most conveniently with a razor blade, consists of two perpendicular, triangularly prismatic troughs which extend along diameters of the surface. In addition to the two troughs, four pie-shaped pieces are cut from the four sectors formed by the cross of the troughs. The complete pattern is cut to the same depth, about 2.3 mm. ($^3/_{32}$ inch). During operation of the apparatus, the face of the pad rests upon the bearing insert and wipes a circular area 2.8 cm. (1.125 inch) in diameter. No weight other than that of the rotor-spindle assembly is applied; in the apparatus now in use this amounts roughly to 2.7 kg. (6 pounds). As the machine operates, the oil on the surface of the bearing is swept outward through the troughs in the felt by centrifugal force, more oil is then pumped up through the radial and coaxial holes of the anchor and bearing, and thus continual contact is assured between oil and bearing.

An extremely vigorous stirring action is imparted to the oil by means of four aluminum fins located on the periphery of the disk of the rotor (Figure 4, *A*). The fins are placed at equal distances and are pitched at an angle of about 45° in such a manner that the oil is lifted as the rotor travels in the clockwise direction.

Gas Train (Figure 1). Although any gas phase can be swept through the apparatus, dried air is most generally used. The air is passed first through 20° Baumé, aqueous sodium hydroxide, then through concentrated sulfuric acid, and finally through a cartridge of glass wool or cotton to remove any entrained particles. The flow of air is metered by means of a manometric-type flowmeter. From the flowmeter the air passes directly through the top of the reaction vessel by means of the combined thermocouple well and air inlet. The air simply passes through the vapor space and is not bubbled through the oil; efficient contact between air and oil is assured by the vigorous agitation of the oil supplied by the rapid stirring. The air passes from the reaction chamber through the top of the reactor, along the shaft of the rotor. The rate of air flow generally used, 600 ml. per minute, allows a complete change of atmosphere approximately once each minute.

Mounting Accessories (Figure 2). For simplicity and convenience the corrosion and stability apparatus employs a conventional, bench-type drill press. Power is furnished by means of a $^1/_3$-hp. motor. Speed of revolution of the rotor is determined by the characteristics of the motor and reduction pulleys provided as standard features of the equipment. For standard testing, a speed of 1310 r. p. m. is used. However, variation has been investigated at 660 and 2410 r. p. m. Constancy of rotation is readily maintained within satisfactory limits (± 1 per cent at 1310 r. p. m.) and is checked periodically by means of a tachometer.

Table I. Standard Conditions for Corrosion and Stability Tests on Lubricants of SAE-10 and 30 Grades

SAE grade of lubricant	10	30
Designation of test	C and S-10	C and S-30
Temperature (both oil and bearing), ° F.	363	363
Duration of test, hours	3	5
Oil charge, grams	225	225
Rotor speed, r. p. m.	1310	1310
Rate of air venting, ml. per minute	600	600
Bearing insert		
Type	Cu-Pb	Cu-Pb
Total area, sq. cm.	7.89	7.89
Area wiped, sq. cm.	6.17	6.17
Ratios		
Total area of bearing per gram of oil, sq. cm.	0.0351	0.0351
Wiped area of bearing per gram of oil, sq. cm.	0.0274	0.0274

Heat is supplied to the vapor bath by means of a Chromolax ring heater of 750-watt capacity. The power supply to the heater is controlled by a Varitran. Variation of heat output facilitates more rapid heating up and the wide range of heat available increases the range of temperature at which operation may be conducted. The apparatus has been run at temperatures ranging from room temperature to 218° C. (424° F.).

A simple steel framework, which can be of any convenient design, supports the reaction vessel and holds it from slipping under the effect of the high speed rotation. It has been found expedient to insulate the reaction vessel by wrapping it with asbestos paper. More efficient utilization of heat is thus realized, as well as the elimination of erratic and localized overheating or subcooling. The top of the reaction chamber is held firmly in place by a gentle pressure exerted through a rubber cushion and a Transite ring which is bolted to the steel framework by means of four bolts with wing nuts.

The Wiping Action. Whereas in other features the corrosion and stability apparatus does not depart markedly in principle from earlier techniques, the nature of its felt wiping action on the bearing surface is distinctive. In agreement with Clower (*3*), who reported that a leather wiping action improved the reproducibility of corrosion measurements of this type, the felt wiping action has been found to contribute satisfactory precision. A felt wiping action is, of course, completely artificial in terms of service. The conditions existing at the bearing-journal site of an engine have been reproduced in the thrust bearing corrosion machine (*10*) which permits the study of corrosion of a loaded bearing under conditions essential to hydrodynamic lubrication. Loading the bearing in the glass equipment of the corrosion and stability test is impossible and hence the new apparatus does not realize this fundamental aim of the thrust bearing corrosion machine. The wiping action of the corrosion and stability apparatus is designed to remove the products of corrosion in analogy to the action of the journal in an engine. It has been repeatedly observed that it does not interfere with the deposition on the bearing of lacquer films by oils which are noncorrosive in an engine, presumably because of the protection afforded the bearing by the deposition of the products of oil oxidation.

Operation of the Corrosion and Stability Apparatus

Conditions of Test. In Table I are listed the conditions of test which have been usefully employed in the evaluation of the potential service performance of SAE-10 and 30-grade motor oils. These conditions were selected as those which permitted satisfactory correlation between the laboratory test and the 36-hour Chevrolet engine test (*1*). Subsequently, these tests have been found capable of predicting with reasonable accuracy the behavior of lubricants in the Standard Caterpillar and General Motors Diesel engine tests (*2*, *4*).

A standard corrosion and stability test is conducted under the conditions listed in Table I in accordance with the following simple procedure:

1. The bearing surface (Figure 4, *D*) is polished successively on several emery papers, the final polishing being given with 4/0 paper. It is then washed with a light naphtha and acetone, dried, and weighed to 0.1 mg.
2. After insertion into the recess of the reaction chamber of the felt cushioning pad and the aluminum anchor (Figure 4, *B* and *C*), the heat-transfer medium is brought to boiling.
3. When the vapors of the heat medium are observed in the

Figure 4. Metal and Felt Equipment

A. Aluminum rotor
B. Felt cushioning pad
C. Aluminum anchor
D. Bearing insert (copper-lead)
E. Felt wiping pad

TABLE II. CORRELATION OF C AND S-30 WITH THE 36-HOUR CHEVROLET ENGINE TEST

(On the basis of the four SAE correlation oils of 30-viscosity grade)

Property		SAE Correlation Oil B-1	SAE Correlation Oil B-2	SAE Correlation Oil B-3	SAE Correlation Oil B-4
Bearing weight loss, mg. per sq. cm.	C and S-30	0.81 ± 0.15 (19%)	6.4 ± 0.2 (3.1%)	1.44 ± 0.04 (2.8%)	0.54 ± 0.07 (13%)
	Engine test	16 ± 6	54 ± 40	14 ± 6	10 ± 9
	Rating, C and S-30	2	4	3	1
	Rating, engine	3	4	2	1
Increase in neutralization No., mg. of KOH per gram	C and S-30	0.8 ± 0.15 (19%)	4.8 ± 0.4 (8.3%)	1.20 ± 0.04 (3.3%)	2.6 ± 0.1 (3.7%)
	Engine test	2.3	5.3	1.6	4.6
	Rating, C and S-30	1	4	2	3
	Rating, engine	2	4	1	3
Increase in viscosity at 100° F., S. U. seconds	C and S-30	68 ± 12 (18%)	160 ± 14 (8.8%)	24 ± 2 (8.4%)	287 ± 19 (6.6%)
	Engine test	490	373	91	1040
	Rating, C and S-30	2	3	1	4
	Rating, engine	3	2	1	4
Naphtha-insolubles, % by weight	C and S-30	0.00–0.01	0.01–0.02	0.00–0.01	1.17 ± 0.01 (0.9%)
	Engine test	1.72	0.78	0.45	2.81
	Rating, C and S-30	–	–	–	4
	Rating, engine	3	2	1	4
Chloroform-solubles, % by weight	C and S-30	< 0.01	< 0.01	0.00–0.01	1.16 ± 0.01 (0.9%)
	Engine test	1.38	0.48	0.12	2.57
	Rating, C and S-30	–	–	–	4
	Rating, engine	3	2	1	4
Increase in Conradson carbon residue, % by weight	C and S-30	0.17	0.92 ± 0.8 (8.7%)	0.34 ± 0.04 (12%)	1.02 ± 0.03 (2.9%)
	Engine test	2.16	2.25	1.29	2.56
	Rating, C and S-30	1	3	2	4
	Rating, engine	2	3	1	4

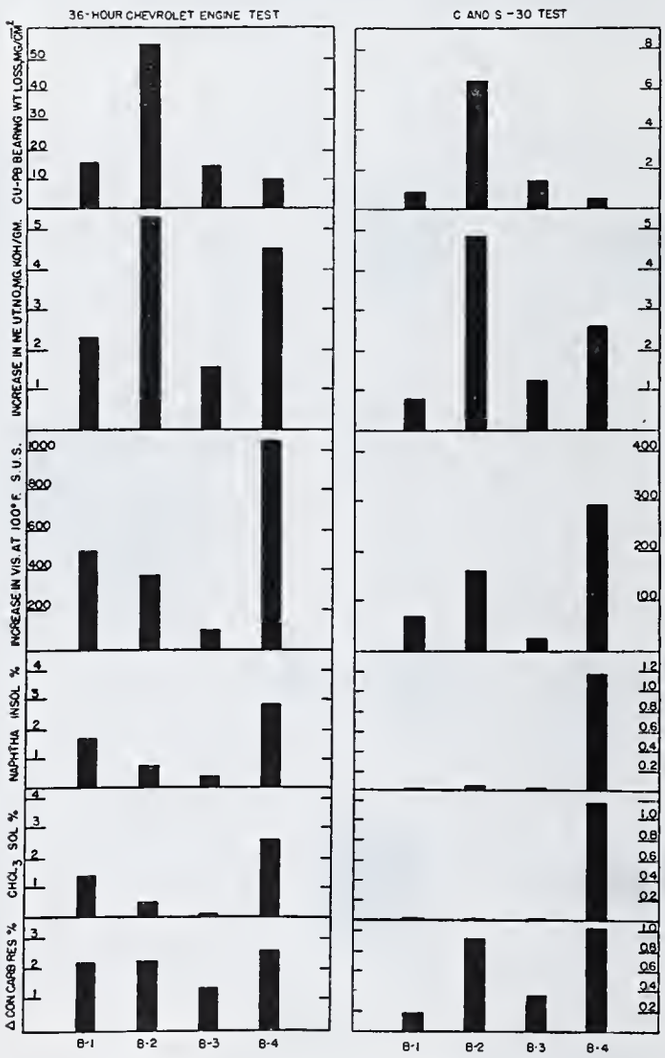

FIGURE 5. CORRELATION DATA BETWEEN C AND S-30 AND 36-HOUR CHEVROLET ENGINE TEST

reflux condenser, 225 grams of the test oil are introduced into the reaction chamber.

4. The oil is judged to be sufficiently heated for introduction of the bearing when vapors are again observed in the reflux condenser. The weighed bearing is then fitted into position. In the case of opaque oils, a Nessler tube is conveniently employed as a spyglass, facilitating visual observation when the closed end is held down against the anchor.

5. After the bearing has been put into place, the apparatus is completely assembled. The rotor in which a fresh felt wiping pad has been placed is rested on the bearing (Figure 4, *A* and *E*), and the cover (Figure 3, *B*), rubber cushioning ring, and Transite clamping ring are assembled and clamped into position with the four wing nuts (Figure 2). The shaft of the rotor is chucked into the drill-press spindle and the rotor is gently lowered until it rests on the bearing. At this point the motor is started to test the apparatus; if everything is in order, operation will proceed smoothly. Occasionally the wiping pad will become displaced from its recess in the rotor; this can be detected when the stirring mechanism is started and can be immediately rectified. The combined thermocouple well and air inlet is set into place, and, to avoid freezing, its ground-glass joint is lubricated with the oil under test. The stirrer should not be set into motion with the rotor raised, since the fins may not clear the thermocouple well and breakage will result.

6. A few minutes, about 10 to 15, are allowed after assembling the apparatus for the oil to come back to temperature after cooling during exposure to atmosphere during the introduction of the bearing. Again this can be judged by appearance of reflux in the condenser. Temperature readings are taken on a Leeds & Northrup indicating potentiometer, the thermocouple having been set in place. To obtain representative temperature measurements, stirring should be in operation, since the thermocouple well may not be sufficiently immersed in the oil otherwise. When reflux is again well up in the condenser, the air flow is started and adjusted to the desired rate by means of the calibrated manometric flowmeter, the stirring started, and zero time recorded. The equilibrium temperature is usually attained in from 3 to 5 minutes. Finally, the suction is supplied to the exit from the reaction chamber (by water aspirator) to prevent dispersing into the laboratory the fumes of deteriorating oil.

7. Normally very little attention is required by the corrosion and stability apparatus during the course of the run. Routine checks are made periodically of the temperature, air flow, and water flow through the condenser and any necessary adjustments made.

8. At the termination of a run, the supply of heat to the vapor bath is discontinued, the air flow is stopped, and the suction which has drawn off vapors from the reaction vessel is cut off. However, flow of cooling water through the condenser is allowed to continue until the heat medium has cooled well below boiling.

TABLE III. CORRELATION OF C AND S-10 WITH THE 36-HOUR CHEVROLET ENGINE TEST

(On the basis of the four SAE correlation oils of 10-viscosity grade)

Property		SAE Correlation Oil B-11	SAE Correlation Oil B-12	SAE Correlation Oil B-13	SAE Correlation Oil B-14
Bearing weight loss, mg. per sq. cm.	C and S-10	0.79 ± 0.02 (2.5%)	1.7 ± 0.2 (12%)	0.8 ± 0.2 (25%)	0.24 ± 0.05 (21%)
	Engine test	12 ± 3	50 ± 28	8 ± 3	2 ± 1
	Rating, C and S-10	2	4	2	1
	Rating, engine	3	4	2	1
Increase in neutralization No., mg. of KOH per gram	C and S-10	0.9 ± 0.1 (11%)	2.8 ± 0.2 (7.1%)	1.25 ± 0.05 (4.0%)	1.7 ± 0.3 (18%)
	Engine test	1.8	6.4	1.95	4.5
	Rating, C and S-10	1	4	2	3
	Rating, engine	1	4	2	3
Increase in viscosity at 100° F., S. U. seconds	C and S-10	24 ± 3 (12%)	38 ± 5 (13%)	28 ± 2 (7.1%)	23 ± 6 (26%)
	Engine test	316.3	198.1	47.1	162.3
	Rating, C and S-10	1	4	3	1
	Rating, engine	4	3	1	2
Naphtha-insolubles, % by weight	C and S-10	0.04 ± (< 0.005)	0.32 ± 0.02 (6.3%)	0.26 ± 0.06 (23%)	0.62 ± 0.02 (3.2%)
	Engine test	2.42	1.16	0.80	1.95
	Rating, C and S-10	1	3 (2)[a]	2 (1)[a]	4 (3)[a]
	Rating, engine	4	2 (2)[a]	1 (1)[a]	3 (3)[a]
Chloroform-solubles, % by weight	C and S-10	0.015 ± 0.005	0.27 ± 0.01 (3.7%)	0.22 ± 0.02 (9.1%)	0.58 ± 0.04 (6.9%)
	Engine test	1.68	0.49	0.18	1.89
	Rating, C and S-10	1	3 (2)[a]	2 (1)[a]	4 (3)[a]
	Rating, engine	3	2 (2)[a]	1 (1)[a]	4 (3)[a]
Increase in Conradson carbon residue, % by weight	C and S-10	0.12 ± 0.02 (17%)	0.375 ± 0.005 (1.3%)	0.12 ± 0.04 (33%)	0.4 ± 0.1 (25%)
	Engine test	2.26	1.89	0.95	1.67
	Rating, C and S-10	1	3	1	4
	Rating, engine	4	3	1	2

[a] Figures in parentheses represent relative ratings if oil B-11 is eliminated from consideration.

The apparatus is disassembled immediately, and the oil is siphoned from the reaction vessel. These manipulations are made immediately at the completion of a run, since it is considered important to keep at a minimum the "overexposure" of the bearing to the hot deteriorated oil.

9. After removal from the apparatus, the bearing insert is washed in solvents as it was prior to its initial weighing. Adhering particles of sludge are removed by gentle swabbing with cotton, but no abrasion severe enough to cause erosion of metal is employed. When clean, the surface of the bearing is examined visually for evidence of erosion or uneven wiping. Erosion is indicated by deep concentric scratches in the surface, whereas uneven wiping results in polished areas unevenly distributed on the "high" areas of the surface. When dry, the bearing is reweighed to evaluate the extent of corrosion which has occurred during the run.

10. The aged oil is subjected to analysis for properties which are regarded conventionally as measures of extent of deterioration—e. g., neutralization number, saponification number—increase in viscosity, and naphtha-insolubles.

CLEANING THE APPARATUS. The apparatus is thoroughly cleaned after each run. While still warm, the reaction vessel is first swabbed out with dry cotton. Kerosene is then introduced and the walls of the vessel are briskly rubbed with cotton. Successive washes then follow with naphtha and acetone until the solvents remain uncolored. The cover, motor, and anchor are likewise washed with kerosene, naphtha, and acetone. Both felt cushioning pad and felt wiping pad are discarded after each run.

Occasionally it is necessary to employ more drastic cleaning procedures. The metal parts can be abraded with steel wool, care being taken to rinse off all metal particles after such treatment. Steel wool is never used on the reaction vessel; rather a prolonged exposure to bichromate-sulfuric acid cleaning solution is employed. This can be effectively accomplished by filling the vessel with the acid and bringing the vapor bath to reflux.

Experimental Results

In their development the standard corrosion and stability tests were correlated with the 36-hour Chevrolet engine test, since considerable data (7) are available from the recent correlation program carried out among several laboratories on this engine test under the supervision of the subcommittees

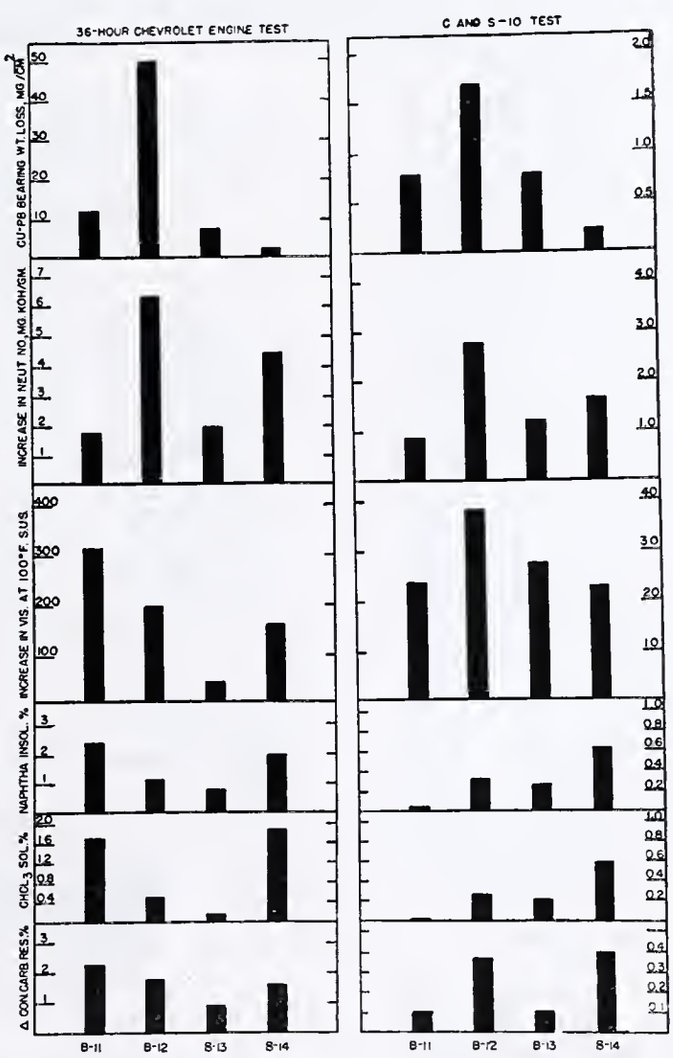

FIGURE 6. CORRELATION DATA BETWEEN C AND S-10 AND 36-HOUR CHEVROLET ENGINE TEST

Table IV. Corrosivity-Zone Schedule for Judging Potential Corrosivity of Lubricants from Results of Corrosion and Stability Tests

Zone	Description	Weight Loss of Cu-Pb Bearing Insert in C and S Test: SAE-10 oils	SAE-30 oils
		Mg. per sq. cm.	
I	Noncorrosive	< 0.5	< 0.75
II	Borderline to mildly corrosive	0.5–1.0	0.75–1.5
III	Mildly corrosive to corrosive	1.0–1.5	1.5–2.0
IV	Excessively corrosive	> 1.5	> 2.0

of Sub-Division B of the Lubricants Division of the Society of Automotive Engineers.

Correlation of C and S-30 with the 36-Hour Chevrolet Engine Test. In Table II and Figure 5 are presented the correlation data for corrosion and stability on 30-grade oils and the 36-hour Chevrolet engine test.

The precision of C and S-30 is strikingly good for this type of test, as concerns both potential corrosivity and oil deterioration. Average error for these data expressed as average deviation from the mean is ±8.2 per cent.

Some reversals will be observed. Oils B-1 and B-3 are reversed by the laboratory test from their relative ratings in the engine in respect to corrosivity, increase in neutralization number, and increase in carbon residue, and B-1 and B-2 are reversed as concerns increase in viscosity. The reversal of B-1 and B-3 in corrosivity is not considered serious, since one third of the participating engine laboratories either rated these oils in the same order as did the C and S-30 or rated them as equal. Further, the averages for all engine laboratories on the corrosivity of these two oils separated them by only 14 per cent of the lower figure (16 mg. per sq. cm. for B-1, 14 mg. per sq. cm. for B-3) which is well within the precision of the engine test. The reversal of B-1 and B-2, in respect to increase in viscosity, occurs again with two oils on which the least differentiation was made by the engine.

The C and S-30 found most difficult the reproduction of engine performance as regards formation of sludge and insoluble bodies in these oils. It is logical that the interfering factors attributable to the fuel, fuel combustion products, dust, and dirt, influential in the engine but absent in the laboratory test, will concentrate their effect in regard to this particular manifestation of deterioration. Nevertheless, it is evident that C and S-30 distinguishes B-4 as the worst oil in regard to naphtha-insolubles, chloroform-solubles, and increase in carbon residue.

Neither the corrosion and stability nor the engine test rates the corrosivity of the oils in the same order as in development of neutralization number. Thus, further evidence is provided of the inadequacy of neutralization number as a criterion of the development of corrosivity.

Correlation of C and S-10 with the 36-Hour Chevrolet Engine Test. In Table III and Figure 6 are presented the correlation data for corrosion and stability on 10-grade oils and the 36-hour Chevrolet engine test.

A relatively high order of precision is maintained in C and S-10; average error for these data is ±13.4 per cent. Ample margin is provided to differentiate readily between oils of reasonably different quality.

The C and S-10 rated oils B-11 and B-13 of equal corrosivity within the precision of the test, whereas the engine test rated B-11 more corrosive than B-13. However, as can be judged from the precision figures for the engine tests (included in Table III) the ranges in corrosivity covered by both oils are sufficient to permit a reversal.

Correlation between laboratory and engine tests on viscosity increase is poor; however, development of naphtha-insolubles, chloroform-solubles, and increase in carbon residue agree satisfactorily with the one exception of oil B-11.

As in the case of the 30-grade oils, here again there is no correlation between development of neutralization number and corrosivity.

Tables II and III indicate that extent of corrosivity and oil deterioration in the corrosion and stability tests falls short of that in the engine test. Standard conditions have been selected deliberately to maintain deterioration in the laboratory test less severe than that in the engine, since the converse might be expected to imperil the validity of the test. Furthermore, it can be judged impossible to find a single set of conditions which would deteriorate all oils to the same extent as does the engine, since the ratio between deterioration in the corrosion and stability apparatus and the engine is not constant for different oils. For example, increase in severity of conditions in the laboratory test sufficient to increase the neutralization number change for oil B-1 to its value in the engine (from 0.8 to 2.3) would be expected to cause the deterioration of oils B-2 and B-3 to exceed that

Table V. C and S-30 Data and Engine Data

[On Group I oils (SAE-40 grade) of S. A. E. Crankcase Oil Stability Research Committee]

	Oil 4	Oil 5	Oil 6	Oil 7	Oil 8	Oil 9
	C and S-30 Data					
Cu-Pb weight loss, mg. per sq. cm.	0.50 ± 0.08	0.68 ± 0.16	2.0 ± 0.3	0.34 ± 0.10	0.93 ± 0.10	−(0.08 ± 0.02)[a]
Relative rank	3	3	6	2	5	1
Δ Neutralization No., mg. of KOH per gram	0.9 ± 0.2	1.75 ± 0.05	2.8 ± 0.7	2.5 ± 0.2	1.9 ± 0.2	−(0.2 ± 0.0)[b]
Relative rank	2	3	5	5	3	1
Δ Saponification No., mg. of KOH per gram	7 ± 2	3.3 ± 0.4	6.8 ± 0.9	5.3 ± 1.7	3.5 ± 1.0	−(0.75 ± 0.05)[b]
Relative rank	5	2	5	4	2	1
Viscosity increase at 100° F., %	20 ± 3	63 ± 4	40.5 ± 0.5	80 ± 10	92 ± 6	2.1 ± 0.0
Relative rank	2	4	3	4	6	1
Naphtha-insoluble, %	0.01–0.02	1.42 ± 0.04	0.03–0.04	1.6 ± 0.2	1.79 ± 0.07	0.04
Relative rank	1	4	2	5	5	2
Over-all relative rank[c]	2	3	5	4	6	1
	Report of S. A. E. Crankcase Oil Stability Research Committee (*8*)					
Over-all relative ratings on basis of test engine	2	3	4	6	4	1
Relative service ratings	2	4	6	3	5	1

Oil No.	Service Description
4	A very good oil but not quite so good as the best
5	Judged not to be a good oil because of sludging and ring sticking tendencies
6	Because of insufficient service data, it is impossible to rate this among other oils. Shown in laboratory to be stable at low temperature, but sticks rings quickly at high temperature.
7	Sludges too quickly to be considered among best oils
8	A very poor oil which had caused every sort of trouble generally considered to arise from lack of stability; a bad sludge maker and ring sticker; unquestionably this oil has the poorest service record of the lot.
9	Free from ring sticking and troubles arising from lack of stability

[a] Gain.
[b] Loss.
[c] This ranking empirically established as follows: corrosivity and naphtha-insoluble rankings weighted each as unity; Δ neutralization No., Δ saponification No., and viscosity increase rankings weighted each as one third.

TABLE VI. C AND S DATA AND ENGINE DATA

[On group III oils (SAE-20 grade) of the S. A. E. Crankcase Oil Stability Research Committee]

	Oil 13		Oil 14		Oil 15	
	C and S Data					
st used	C and S-10	C and S-30	C and S-10	C and S-30	C and S-10	C and S-30
-Pb weight loss, mg. per sq. cm.	0.32 ± 0.00	0.64	0.28 ± 0.05	0.39	2.36 ± 0.02	4.3
Relative rank	1	2	1	1	3	3
Neutralization No., mg. of KOH per gram	2.0 ± 0.2	3.0	0.25 ± 0.05	0.5	5.0 ± 0.6	11.4
Relative rank	2	2	1	1	3	3
Saponification No., mg. of KOH per gram	3.4 ± 0.0	5.6	−(0.6 ± 0.2)[a]	3.7	19 ± 3	28
Relative rank	2	2	1	1	3	3
scosity increase at 100° F., %	46 ± 2	87	2.4 ± 0.0	4.6	53 ± 6	280
Relative rank	2	2	1	1	3	3
phtha-insoluble, %	1.3 ± 0.1	1.7	0.02–0.04	0.02	0.01	0.06
Relative rank	3	3	2	1	1	2
er-all relative rank[b]	2	2	1	1	3	3
	Report of S. A. E. Crankcase Oil Stability Research Committee (9)					
er-all relative ratings on basis of test engines	3		1		2	
lative service ratings	3(close to No. 15)		1(much the best)		2(close to No. 13)	

Oil No.	Service Description
13	An oil generally rated of low stability; in sludge forming and ring sticking, its behavior is not good.
14	An oil which has shown very good oxidation stability characteristics; under both severe and long-time mild conditions stability has been satisfactory in both gasoline and Diesel equipment and engine deposits relatively small.
15	An oil of medium stability; has worked out successfully in a wide field of application, but fails under severe operating conditions.

[a] Loss.
[b] This ranking empirically established as follows: corrosivity and naphtha-insoluble rankings weighted each as ity; Δ neutralization No., Δ saponification No., and viscosity increase rankings weighted each as one third.

tained in the engine. Of course, an interfering factor in is concern is the practice of adding make-up oil during the gine test, a feature which is influential in determining the extent of oil deterioration as measured by the properties of the final oil sample. A more satisfactory expedient would be to measure deterioration as a function of time. This is conveniently achieved in corrosion and stability tests by sampling the oil charge at intervals during a run. The importance of time as a variable influential among the factors affecting oil deterioration and bearing corrosion is discussed in a companion communication (*12*).

DEFINITION OF PERMISSIBLE LIMITS OF CORROSIVITY. From the data of Tables II and III a scale for rating the potential corrosivity of lubricants of these two viscosity grades has been drawn up. It is, of course, recognized that no sharp line of demarkation between noncorrosive and corrosive oils can be made. Judgment is based, rather, on "zones" of corrosivity in accordance with the schedule outlined in Table IV.

EVALUATION IN CORROSION AND STABILITY APPARATUS OF LUBRICANTS CIRCULATED BY S. A. E. CRANKCASE OIL STABILITY COMMITTEE (*8*, *9*). With the conditions of test selected upon the basis of the results obtained on the B oils as described above, various correlation oils selected by the S. A. E. Crankcase Oil Stability Research Committee have been studied in the corrosion and stability apparatus, since they provided further opportunity to ascertain the accuracy with which the laboratory test predicts engine service. Oils of SAE-40 grade (Nos. 4 through 9) and of SAE-60 grade (Nos. 16 through 18) were subjected to conditions of test selected for SAE-30 grade oils (C and S-30); oils of SAE-20 grade (Nos. 13 through 15) were tested under the conditions of both C and S-10 and C and S-30. It was elected to examine these oils under both the milder and more severe conditions, since no set of conditions has been established for 20-viscosity grade oils, because of the lack of engine data of the desired scope as provided in the B oils of the S. A. E. committee.

In Table V are presented the results, both from the engine and C and S-30 studies on the SAE-40 grade oils. In each case the relative ratings as established by the corrosion and stability test agree either equally well or more closely with the service ratings than do the over-all ratings based upon the performance of the oils in the test engines. Oils 5, 7, and 8, which are described as sludging oils in service, tended to developexcessive amounts of naphtha-insolubles in the laboratory test. In regard to corrosivity, if the scale developed for SAE-30 grade oils be employed, distribution of the six oils among the various zones of corrosiveness is as follows:

Zone I	Oils 4, 5, 7, 9	Noncorrosive
Zone II	Oil 8	Borderline to mildly corrosive
Zone III	Oil 6	Mildly corrosive to corrosive

TABLE VII. C AND S-30 DATA AND ENGINE DATA

[group IV oils (SAE-60 grade) of S. A. E. Crankcase Oil Stability Research Committee]

	Oil 16	Oil 17	Oil 18
	C and S-30 Data		
C-Pb weight loss, mg. per sq. cm.	0.72 ± 0.17	0.35 ± 0.11	0.13 ± 0.03
Relative rank	3	2	1
eutralization No., mg. of KOH per gram	1.8 ± 0.6	0.30 ± 0.05	0.1 ± 0.0
Relative rank	3	2	1
aponification No., mg. of KOH per gram	11.4 ± 0.3	5.6 ± 1.1	0.6 ± 0.1
Relative rank	3	2	1
cosity increase at 100° F., %	45 ± 7	9.5 ± 0.5	0–1
Relative rank	3	2	1
phtha-insolubles, %	0.03–0.04	0.02–0.03	0.04
Relative rank	1	1	1
r-all relative rank[a]	3	2	1
	Report of S. A. E. Crankcase Oil Stability Research Committee (9)		
r-all relative rating on basis of test engines	3	2	1
ative service ratings	2	3	1

Oil No.	Service Description
6	An oil of medium stability characteristics; reasonably satisfactory under a fairly wide range of operating conditions but not entirely suitable for all types of operation
7	An oil of low stability characteristics; capable of reasonably satisfactory performance under relatively easy service conditions; distinctly unsatisfactory in high performance engines in moderately severe service
8	An oil of high stability; superior from standpoint of lacquer, sludge, and ring sticking; possesses high stability rating over widest possible service range

This ranking empirically established as follows: corrosivity and naphtha-insoluble rankings weighted each as unity; Δ neutralization No., Δ saponification No., and viscosity increase rankings weighted each as one third.

TABLE VIII. EVALUATION IN CORROSION AND STABILITY TEST OF HEAVY-DUTY ALL-PURPOSE LUBRICANTS

	Oil A	Oil B	Oil C	Oil D	Oil E	Oil F	Oil G	Oil H [c]	Oil I [c]
Viscosity index (Dean and Davis)	100	70	100	80	90	90	90	100	87
SAE viscosity grade	30	30	30	30	30	30	30	10	10
Additive	Inhibitor detergent	Inhibitor detergent	Inhibitor	Inhibitor detergent	None	Inhibitor detergent	Inhibitor detergent	Inhibitor detergent	Inhibitor detergent
Laboratory test	C and S-30	C and S-30	C and S-30	C and S-30	C and S-30	C and S-30	C and S-30	C and S-10	C and S-10
Cu-Pb weight loss, mg. per sq. cm.	1.25 ± 0.03	0.75	0.48	1.7	1.6 ± 0.2	2.0 ± 0.1	1.1 ± 0.1	0.44	1.3 ± 0.1
Final neutralization No., mg. of KOH per gram	1.0 ± 0.0	0.7	0.1	0.7	1.7 ± 0.2	0.55 ± 0.05	0.45 ± 0.05	0.4	0.8 ± 0.2
Final saponification No., mg. of KOH per gram	4.5	4.0	....	....	9.3 ± 0.3	4.0 ± 0.5	3 ± 2	2.8	5 ± 1
Viscosity increase at 100° F., %	7.1	20	....	19	27 ± 1	6.5 ± 1.2	8.1 ± 0.5	4.0	27
Naphtha-insolubles, %	0.01	0.02	....	0.91	0.12 ± 0.04	0.10 ± 0.00	0.06 ± 0.02	0.03	0.07
Corrosivity rating	Zone II, borderline to mildly corrosive	Zone II, borderline to mildly corrosive	Zone I, noncorrosive	Zone III, mildly corrosive to corrosive	Zone III, mildly corrosive to corrosive	Zone IV, excessively corrosive	Zone II, borderline to mildly corrosive	Zone I, noncorrosive	Zone III, mildly corrosive to corrosive
Engine test [a]	1 2	1 2	2	1	3	3	3	3	3
Cu-Pb weight loss, mg. per 1/2 bearing	18	....	....	924	5–50	42–106	12	....	28
Final neutralization No., mg. of KOH per gram	....	.... 0.4	....	....	0.2	0.4	0.3	....	0.6
Final saponification No., mg. of KOH per gram	....	.... 2.4	....	....	....	6.7	5.1	....	4.8
Viscosity increase at 100° F., %	....	.... 4.5 [b]	....	....	5	11	14	....	57
Naphtha-insoluble, %	....	 11	....	....	0.14	0.42	0.49	....	0.77
Corrosivity rating	....	.... 25 [b]	....	....	Tendency to be corrosive	Corrosive	Satisfactory	Satisfactory	Above borderline in corrosivity
	Satisfactory Corrosive	Satisfactory Acceptable but mildly corrosive	Satisfactory	Corrosive					

[a] Engine test designations: 1 Caterpillar Diesel 240-hour test (2)
2 General Motors Diesel 500-hour test (4)
3 Caterpillar 3-A Diesel 120-hour test (2)

[b] Properties of lubricant sampled at 384th hour of 500-hour test.
[c] H and I contained also a pour-point depressant.

Two of the four oils which fall in the non corrosive zone I (oils 5 and 7) nevertheles display considerable tendencies to develop acid as judged by neutralization number. Thei failure to become corrosive may be attribute to deposition of protective films on the bearin surface; this is compatible with their develop ment of high naphtha-insolubles.

Table VI lists the data on the SAE-20 grad oils. The laboratory test rated the three oil in the same order under both the milder an more severe conditions. It is felt, since in th S. A. E. committee's report (*9*) it is emphasize that these oils are moderate-duty lubricants an not designed for heavy-duty service, that th milder conditions of test are more appropriat This is substantiated by the fact that unde the more severe conditions, oil 15 is oxidize more severely in the laboratory test than i the engine. The practice, however, of testin SAE-20 grade lubricants under the condition of C and S-10 is by no means generally em ployed. For heavy-duty, all-purpose oils, it i preferred to apply the more severe condition of test, since there is thus provided a factor c safety compatible with the service the lubr cant may be called upon to render.

Consideration of the order of rating by th corrosion and stability tests reveals that oil 1 is definitely superior to oils 13 and 15, whic is consistent with both the test engine an service ratings. In regard to these latter tw oils, it may be judged that No. 13 would b unsatisfactory from sludging trouble and N 15 affords insufficient protection against corr sion of copper-lead bearings. The relativel great deterioration of oil 15 exhibited by hig neutralization number, saponification numbe and viscosity increase is due probably in pa at least to catalysis by metal removed from th copper-lead bearing. Severe oil oxidation is fr quently observed accompanying high weig loss with this type of bearing. In the absenc of this factor of catalysis, the differentiation o tained in the corrosion and stability tests b tween oils 13 and 15 with respect to stabilit would probably not be so marked. Corrosivit ratings, in accordance with the zone schedul of Table IV for the 20-grade oils, are:

Zone I	Oils 14, 13	Noncorrosive
Zone IV	Oil 15	Excessively corrosive

It may be judged that oil 13 fails to b come corrosive through the protection afford to the bearing surface by the deposition c products of oil oxidation (naphtha-insolubles).

In Table VII are presented the results o tained upon the oils of SAE-60 viscosity grad It will be seen that the corrosion and stabilit test and the test engines rate these three oil in the same order, whereas both show a r versal with respect to the service ratings of oil 16 and 17. This reversal may be due to th selection of insufficiently severe conditions f the laboratory test properly to evaluate lubr cants of this high viscosity grade. It appea likely, however, that greater difference in qualit exists between oil 18 and either of oils 16 an

7 than exists between oils 16 and 17 themselves, and this clearly disclosed by the corrosion and stability test. All ree SAE-60 grade oils rate as noncorrosive (zone I on the hedule for 30-grade oils). Nevertheless, there is sufficient ariation among the corrosivities of the three lubricants r definite individual differentiation well outside the precion of the laboratory test.

Heavy-Duty All-Purpose Lubricants. An application which the corrosion and stability apparatus has been useilly employed is the preliminary examination of lubricants rior to protracted approval tests in heavy-duty type engines. rom the experience gained thus far, it has been judged that e corrosion and stability characteristics of oils can be edicted with reasonable accuracy on the basis of the ne schedules drawn up in Table IV and analysis of aged ls.

Table VIII presents data on several heavy-duty, allrpose lubricants of both SAE-10 and 30 grades which have en evaluated in the corrosion and stability test and an gine test. Excellent agreement will be observed between e corrosivity predicted from the corrosion and stability st and that observed in the engine. In some cases oxidation more severe in the laboratory test than in the engine, as dged by the properties of the final oil sample. That this probably caused by the practice of adding make-up oil during e engine test is indicated by the properties of the sample of B removed from the engine at the 384th hour of the 500ur test. At this point, the properties of the used oil licate greater deterioration than was obtained in the oratory test. It is possible, in general, to obtain a useful d reasonably accurate idea of the stability characteristics an oil from the analysis of the oil aged in the corrosion and bility tests. These used oil properties are furthermore lpful in accounting for the corrosiveness of an oil and in closing inherent weaknesses of lubricants, such as susptibility to catalysis by metals, etc.

Metal Catalysis. In the standard corrosion and stabil- tests, as defined by the conditions of Table I, the only l source of catalysis by metals of the deterioration of the is the copper-lead bearing insert, since aluminum is nerally recognized to be relatively inactive. This constutes definitely a departure from conditions of an engine which exposure to metals, principally iron, is outstanding. owever, the conditions selected as standard were chosen pirically in order to obtain favorable comparison between rosion and stability tests and engine performance. On the s discussed in this paper, this was achieved without inclusn of extraneous metal catalysts. However, ample proion has been made in the design of the apparatus for the lusion of catalysts, and the importance of this factor is ly recognized by the authors. It is visualized that extsion in the scope of the apparatus, as well as improvent in accuracy and hence usefulness of the test, may be ected through the addition of catalysts.

The utility of the corrosion and stability tests has been ablished through the experience gained in the examination such lubricants as are described in Table VIII. Despite failure to correlate exactly with the engine behavior of oils upon which extensive engine data are available, the ent of correspondence has been sufficient to inspire conence in the laboratory test. By submitting lubricants, engine performances of which have not been evaluated, a corrosion and stability test appropriate to viscosity grade, s possible to determine whether the more expensive and e-consuming engine test can profitably be run.

Summary and Conclusions

This communication describes the corrosion and stability aparatus and presents results obtained with it in its application as a tool useful in predicting the potential service performance of lubricants. Correlation of the results of the test with behavior of oils in various types of engines is considered to be generally satisfactory, since no laboratory test of this type can take into account all the factors operative in the engine. The corrosion and stability apparatus is simple, inexpensive, and durable; the conditions of test are such that convenient application is possible for either research or control purposes. In design, the apparatus is broadly flexible and, therefore, as variations are made in engines and their conditions of operation, it should be possible to modify the test to compensate for them.

The corrosion and stability apparatus has been extensively employed in these laboratories in the study of the factors affecting bearing corrosion. The effects of variation of the important physical factors, such as time, temperature, catalysis, concentration of reactants, and type of bearing alloy, have been followed in the corrosion and stability apparatus. Discussion of this work is presented in a companion communication (*12*).

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It is also desired to express appreciation to the Society of Automotive Engineers for permission to use unpublished data on the oils used in the correlation program of the S. A. E. Crankcase Oil Stability Research Committee.

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Coprecipitation of Chromate with Barium Sulfate

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The coprecipitation of chromate with barium sulfate has been studied quantitatively under carefully controlled conditions. Acidity, rate of precipitation, ratio of chromate to sulfate, and time of digestion were varied in turn.

The results obtained support the view that for the most part the chromate coprecipitated is probably in the form of barium chromate in mixed crystals with barium sulfate.

THE error caused by coprecipitation of dissolved substances with barium sulfate in gravimetric analysis has been widely recognized. Studies of coprecipitation of various anions have been reported recently (*5*, *6*), but chromate has been omitted from detailed consideration. Kolthoff (*2*) and Manov and Kirk (*3*) have considered briefly the coprecipitation of chromate with barium sulfate during volumetric analysis for sulfate by the method originally devised by Andrews (*1*). Their methods were, however, not precise, since conditions were not rigidly controlled and the amount of chromate coprecipitated was estimated by analyzing the filtrate rather than the precipitate. The authors' preliminary experiments showed that inconsistent results are obtained unless conditions are carefully controlled.

In neutral solution barium chromate is precipitated nearly quantitatively whether or not sulfate is present. Barium sulfate ordinarily is precipitated from acid solutions, however, in the presence of a slight excess of barium ion. Willard and Schneidewind (*7*) in presenting their widely accepted method of analysis for sulfate in chromium plating baths, state: "Barium chromate is so insoluble in dilute acids that it almost invariably contaminates the barium sulfate, and if sufficient acid is used to prevent this, incomplete precipita tion occurs." This statement is an oversimplification, sinc barium chromate will not precipitate by itself, even in th rather dilute acid solutions from which it is customary t precipitate barium sulfate, unless the chromate concentra tion is high. Coprecipitation of chromate with barium sul fate from acid solution is presumably due for the most par to the formation of mixed crystals, since barium sulfate an barium chromate are isomorphous. Other causes may ac count for a small proportion of the coprecipitation of chro mate, perhaps as sodium chromate or dichromate.

The coprecipitation of barium chromate with barium sul fate in acid solution is unusual in that the concentration o chromate ion is very low, although there is a "reservoir" o potential chromate ions in the solution, since in the presence o acid, equilibrium exists among CrO_4^{--}, $HCrO_4^-$, and $Cr_2O_7^-$ ions in aqueous solution:

$$2CrO_4^{--} + 2H_3O^+ \rightleftharpoons 2HCrO_4^- + 2H_2O \rightleftharpoons Cr_2O_7^{--} + 3H_2O$$

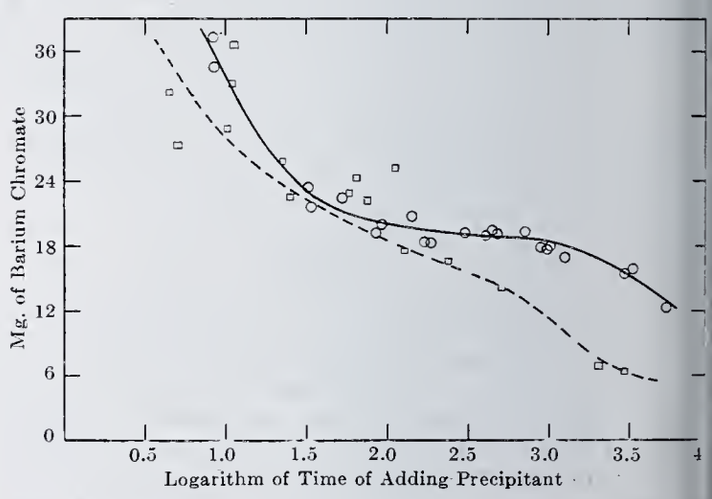

FIGURE 2. VARIATION OF COPRECIPITATION WITH LOGARITH OF TIME IN SECONDS OF ADDING PRECIPITANT
○. Without stirring □. With stirring

The greater the concentration of hydrogen ion, the lower the concentration of chromate ion. As barium dichromate not isomorphous with barium sulfate, the extent of mixe crystal formation is largely dependent on the acid concen tration. Other factors which might be expected to affec the amount coprecipitated, whether from mixed cryst formation or from other causes, are rate of addition of the pre cipitant, concentration of chromate, and length of time c digestion of the precipitate.

This paper reports the results of a study of the extent t which chromate is coprecipitated with barium sulfate, on condition at a time being altered while the others were hel constant.

Procedure

The chromate in a given barium sulfate precipitate was dete mined by the method described by Meldrum, Cadbury, an Lucasse (*4*). The precipitate was fused with sodium carbona and the melt allowed to cool and then disintegrated with wate The mixture was filtered and the filtrate acidified and analyze iodometrically for chromate.

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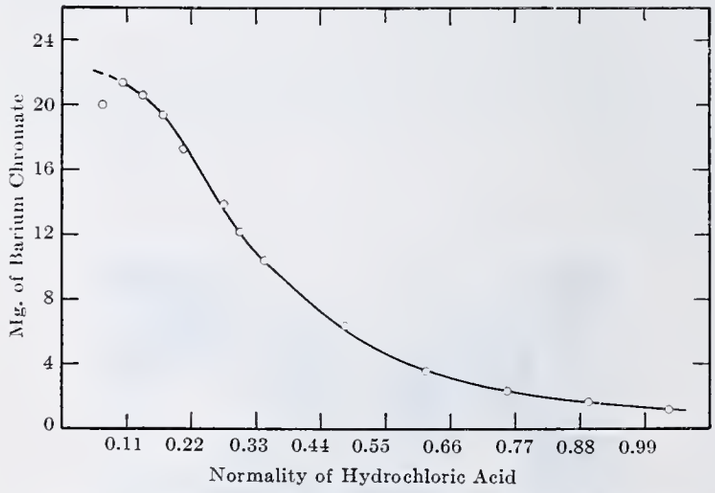

FIGURE 1. VARIATION OF COPRECIPITATION WITH CONCENTRATION OF ACID

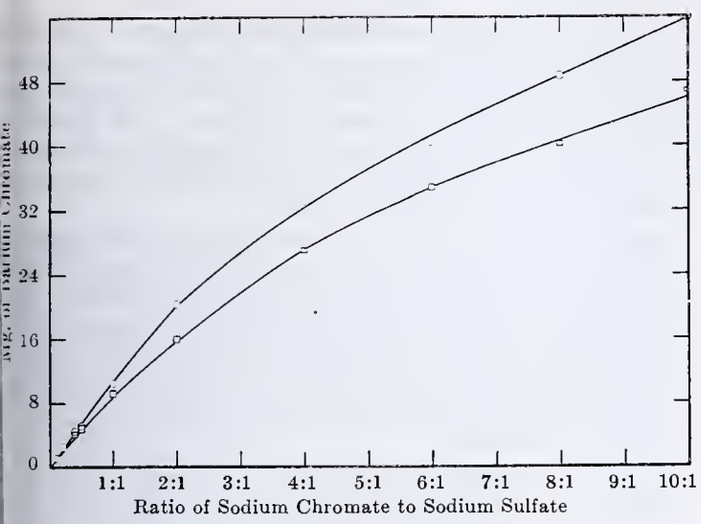

FIGURE 3. VARIATION OF COPRECIPITATION WITH CONCENTRATION OF SODIUM CHROMATE, SODIUM SULFATE HELD CONSTANT

○. Amount of acid constant
□. Excess acid constant

The reagents and solutions were prepared as described in the aper previously referred to (*4*).

Sodium sulfate solution containing about 0.3 gram of sodium ılfate in 25 ml. was introduced from a 25-ml. pipet into a 200-ml. rlenmeyer flask, and the desired amounts of dilute hydroıloric acid, water, and sodium chromate solution were added. he flask was fitted with a reflux condenser, in the top of which as placed a cork carrying a glass tube of 1-mm. bore. This diffusion tube" was intended to prevent loss of vapor and conequent change of concentration. Its effectiveness was demonrated by refluxing an acid solution for 3 hours, cooling, and trating a measured sample with standard base. Comparison ith similar samples which had not been refluxed showed no etectable change in acid concentration during boiling.

The flask with its contents was placed on a hot plate and eated to boiling, the diffusion tube was removed, and 25 ml. of arium chloride solution of such concentration as to supply a ight excess of barium ion were added from a dropping funnel rough the condenser. The diffusion tube was replaced and the recipitate was digested by boiling gently on the hot plate. The ixture was then cooled and filtered, the paper burned off, and ıe precipitate weighed and treated as described above to deterine the amount of coprecipitated chromate.

VARIATION OF ACID CONCENTRATION. Twenty-five-milliliter rtions of equimolar solutions of sodium sulfate and sodium ıromate were mixed, and the desired amount of hydrochloric id was added from a buret, together with enough water to make e volume of acid plus water 75 ml. The total volume of the lution, after addition of 25 ml. of precipitant, was thus 150 ml. he precipitant was added dropwise through a fine capillary ıbe, so that its addition took from 15 to 19 minutes. With this ow rate of addition boiling, with its resultant stirring effect, as continuous. The mixture was digested for 3 hours, after hich the hot plate was turned off. The flask was allowed to ol for 45 minutes, then removed from the hot plate, and cooled pidly in ice water to 20° C. The precipitate was filtered off ıd treated as described above.

All factors were thus kept constant except the acid concentraon, which could be varied at will. Tests showed that even in ıe most concentrated acid solutions used no oxidation of hydroıloric acid took place.

VARIATION OF RATE OF ADDITION OF PRECIPITANT. Equal lumes (25 ml.) of equimolar solutions of sodium sulfate and dium chromate were again used, together with 15 ml. of 2.068 *N* id and 60 ml. of water. When the solution was boiling eadily, 25 ml. of precipitant were added through the condenser before, the time of addition being noted. When addition was w, as in the experiments in which the acid concentration was ıried, gentle boiling produced adequate stirring. When the ol precipitant was added rapidly, however, boiling stopped for while. Two series of runs were made: one without auxiliary irring, and one using a long stirring rod operated through the ndenser, turned by a low-speed motor. The other factors, ch as time of digestion, were kept constant as before.

VARIATION OF CHROMATE CONCENTRATION. The quantity sodium sulfate was held constant as before, but the ratio of dium chromate to sodium sulfate was varied over the range m 1 to 10 to 10 to 1 while other conditions were held constant. Since the amount of acid necessary to convert chromate into dichromate (assuming completeness of reaction) increased as the concentration of sodium chromate was increased, "constant acid concentration" was defined in two different ways: constant total acid, and constant excess acid. No determinations were made at constant pH.

The first set of determinations was carried on with a constant amount of acid regardless of the amount of chromate present: 25 ml. of 2.068 *N* acid were more than sufficient, according to the stoichiometric equation, to convert all the chromate into dichromate.

In the second set of experiments, 25 ml. of 2.068 *N* acid were added in addition to the amount calculated for the conversion of chromate into dichromate. Other conditions were the same as when the acid concentration was varied.

VARIATION OF TIME OF DIGESTION. Equal volumes (25 ml.) of equimolar solutions of sodium sulfate and sodium chromate were mixed, 15 ml. of 2.068 *N* acid and 60 ml. of water were added, and the precipitant was added slowly to the hot solution as before. The period of digestion was considered to begin as soon as the last drop of precipitant was added. At the end of the time of digestion, the mixture was cooled in ice water as rapidly as possible to 20° C., and the precipitate was filtered off and analyzed.

Results and Discussion

The results are plotted in Figures 1 to 4, in each of which the ordinates represent the number of milligrams of chromate, calculated as barium chromate, coprecipitated with about 0.5 gram of barium sulfate (0.502 gram in Figure 1, 0.546 gram in Figures 2, 3, and 4). Each point on Figures 1 and 4 represents the average of two or more determinations.

When the acid concentration is varied (Figure 1) the amount of chromate coprecipitated decreases regularly with increasing acid concentration. Coprecipitation drops off nearly to zero at high acid concentrations, but from the analytical point of view this is no advantage, since barium sulfate becomes appreciably soluble when the acid concentration exceeds 0.5 *N*. In very dilute acid, the results are inconsistent.

When rate of addition of precipitant is varied (Figure 2), the results are less regular, but the general trend is definite: the more rapidly the precipitant is added, the greater is the amount of chromate coprecipitated. Stirring reduces the amount only a little.

When the chromate-sulfate ratio is varied (Figure 3), the results are what would be expected: the greater the ratio, the more chromate is coprecipitated; and the more acid there is present (lower curve), the less chromate is coprecipitated.

From Figure 4, showing variation in time of digestion, it is clear that in order that coprecipitation may not be too great, the mixture must be digested for an hour or more. Times longer than 3 hours have little additional effect. The most

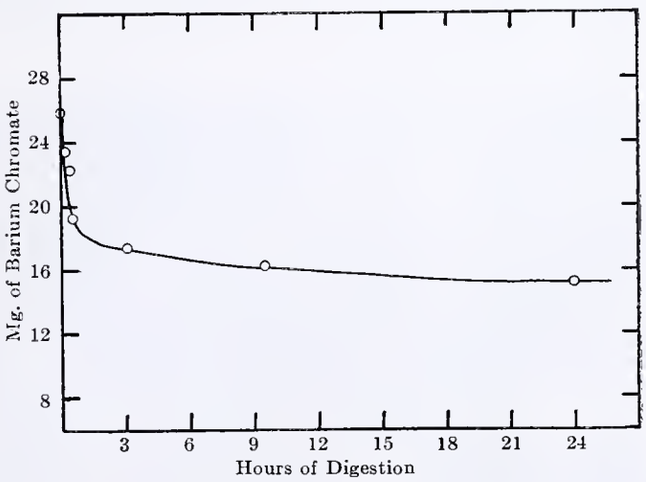

FIGURE 4. VARIATION OF COPRECIPITATION WITH TIME OF DIGESTION

Table I. Results with Varying Times of Digestion

Time of Digestion	Total Weight of Ppt.	$BaCrO_4$ Found	$BaSO_4$ Found	Na_2SO_4 Found	Error
Hours	*Gram*	*Gram*	*Gram*	*Gram*	*Gram*
0	0.5734	0.0275	0.5459	0.3323	−0.0004
0	0.5678	0.0245	0.5433	0.3307	−0.0020
1/6	0.5666	0.0237	0.5429	0.3304	−0.0023
1/6	0.5693	0.0233	0.5460	0.3324	−0.0003
1/3	0.5672	0.0222	0.5450	0.3318	−0.0009
1/3	0.5657	0.0224	0.5433	0.3307	−0.0020
1/2	0.5592	0.0185	0.5407	0.3291	−0.0036
1/2	0.5600	0.0200	0.5400	0.3287	−0.0040
3	0.5640	0.0179	0.5461	0.3324	−0.0003
3	0.5640	0.0171	0.5469	0.3328	+0.0001
10	0.5637	0.0168	0.5469	0.3328	+0.0001
10	0.5628	0.0159	0.5469	0.3328	+0.0001
24	0.5607	0.0152	0.5455	0.3321	−0.0006
24	0.5615	0.0153	0.5462	0.3325	−0.0002

likely explanation for this is that most, if not all, of the coprecipitation remaining after 3 hours is due to mixed crystal formation. However, without additional evidence, such as x-ray examination of the crystals themselves, this interpretation cannot be considered as proved. The excess above the minimum is probably due to one or more of the other causes of coprecipitation.

The data from which Figure 4 is plotted are shown in columns 1 and 3 of Table I. (Similar tables for the other graphs are unnecessary.)

Assuming that all the chromate in the precipitate is in the form of barium chromate, the weight of barium sulfate is found by subtracting the weight of barium chromate from the weight of the precipitate. In column 3 is given the amount of chromate found from the titration, converted to the equivalent amount of barium chromate. This is subtracted from the total weight of the precipitate, column 2, to obtain the weight of barium sulfate, given in column 4. This in turn is multiplied by the gravimetric factor, 0.6086, to convert to sodium sulfate, given in column 5. The difference between this and 0.3327, the number of grams of sodium sulfate taken each time, is called "error", listed in column 6.

Since the errors are small when the precipitate is digeste for 3 hours or more, it is apparent that the assumptions mad are valid. When the precipitate is not digested long enoug the errors are larger and negative in sign. This would b accounted for if the chromate, calculated as barium chromat were actually coprecipitated in part as sodium chromat sodium dichromate, or chromic acid.

Similar analysis of the data from which the other graph were plotted indicated that when the precipitate is forme under the best analytical conditions, with slow addition of th precipitant and sufficiently long digestion, chromate is co precipitated as barium chromate. Appreciable negativ errors at high acid concentrations are in accord with th well-known fact that barium sulfate is somewhat soluble i strongly acid solutions.

Since the investigations of Nichols and Smith (*5*) and c Schneider and Rieman (*6*) were made under conditior different from the authors', no direct comparison is possibl between the amount of chromate coprecipitated and th amounts of other anions as found by these workers. The investigations were made in neutral solutions, for exampl whereas this investigation was of necessity in acid solutio since chromate would precipitate virtually completely in th presence of excess barium ion in neutral solution. Othe factors making a direct comparison impossible inclu differences in concentration and in rate and temperature precipitation.

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Moderately Large Extractor-Percolator Assembly

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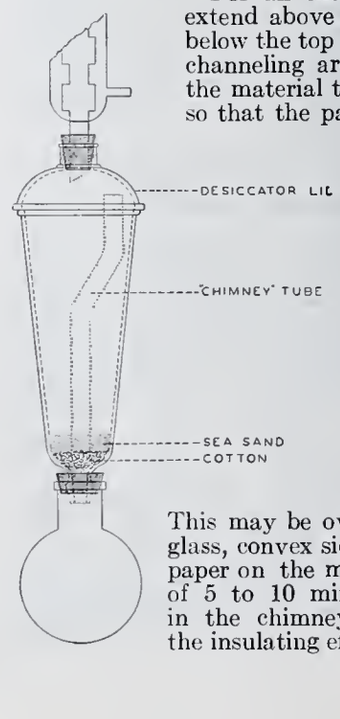
MANY large-scale Soxhlet-type extractors have been reported in the literature, yet a simple modification of the original continuous Soxhlet extractor has proved to be the most useful in the author's laboratory when a moderately large extraction is to be made. This modified extractor can also be used as a percolator of the automatic type, the choice of function depending only upon the method used in packing the extraction chamber.

A diagram of the apparatus is shown. The upper rim of a standard 2-gallon glass percolator is ground flat with 280-mesh Carborundum powder, so that a desiccator lid fits it snugly. If both the percolator rim and the desiccator lid are given a final grinding with 800- or 1000-mesh Carborundum powder, no lubrication of the joint will be required even with petroleum ether or diethyl ether as extracting solvent. This final grinding, however, is time-consuming and, for most purposes, unnecessary. A piece of glass tubing, 15 mm. in diameter, is irregularly flanged at one end, and bent as indicated in the diagram so as to avoid backdrip directly into the boiler. This tube, or "chimney", carries the solvent vapors from the boiler to the condenser. A cotton plug is packed around this chimney where it contacts the neck of the percolator; for percolation, this plug is all that is required. If true extraction is desired, however, a 2.5-cm. (1-inch) layer of clean sea sand or other inert, finely divided material is placed on top of this cotton plug in order to retard the rate of downflow of solution. Finally, on the neck of the percolator is placed a rubber stopper of the appropriate size to fit the boiler, and an efficient condenser is attached to the upper opening of the desiccator l by means of another rubber stopper.



For an extraction, the packing should n extend above a level which is 5 cm. (2 inche below the top of the chimney tube. Ordinaril channeling around this tube does not occur the material to be extracted is 40-mesh or fine so that the packing operation requires no gre care. The rate of boiling the solvent is so regulated th a layer of liquid about 1. cm. (0.5 inch) deep remains top of the material being e tracted.

For a percolation procedur the packing plug consists cotton only, and the rate boiling is immaterial, so lor as the rate of percolation is n exceeded.

Occasionally, the drip fro the condenser tends to dig hole in the top layers of t marc even in an extractio This may be overcome by placing a small wat glass, convex side down, or a small piece of filt paper on the marc. After an initial warming-u of 5 to 10 minutes, the solvent that condens in the chimney is remarkably small, because the insulating effect of the marc packed around i

Quantitative Determination of Benzene

In the Presence of Cyclohexane

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A RECENT paper (*1*) by Corson and Brady reported the development of a method for the determination small amounts of benzene in cyclohexane by measuring e heat of reaction of benzene with a nitrating acid. The per brought out two useful points: the composition of the trating acid producing the best results, and the variation reaction heat due to changes in the initial or starting tem- rature of the reaction.

By making a number of changes, both in the method and the design of the reaction apparatus, it has been possible develop a more rapid and practical procedure.

Apparatus and Reagents

When using the method described by Corson and Brady), which required removal of the stirring device and in- rtion of a thermometer whenever a temperature reading s necessary, some difficulty was encountered in manipula- n of the apparatus. This was especially noticeable in perimental work when it was necessary to take temperature readings a minute or two apart. In addition, an error was introduced when the room temperature changed between any two readings, because both the stirrer and the thermometer, when removed, changed to room temperature and were then replaced in the vacuum flask. Then, too, each time one or the other was removed some of the solvent was lost.

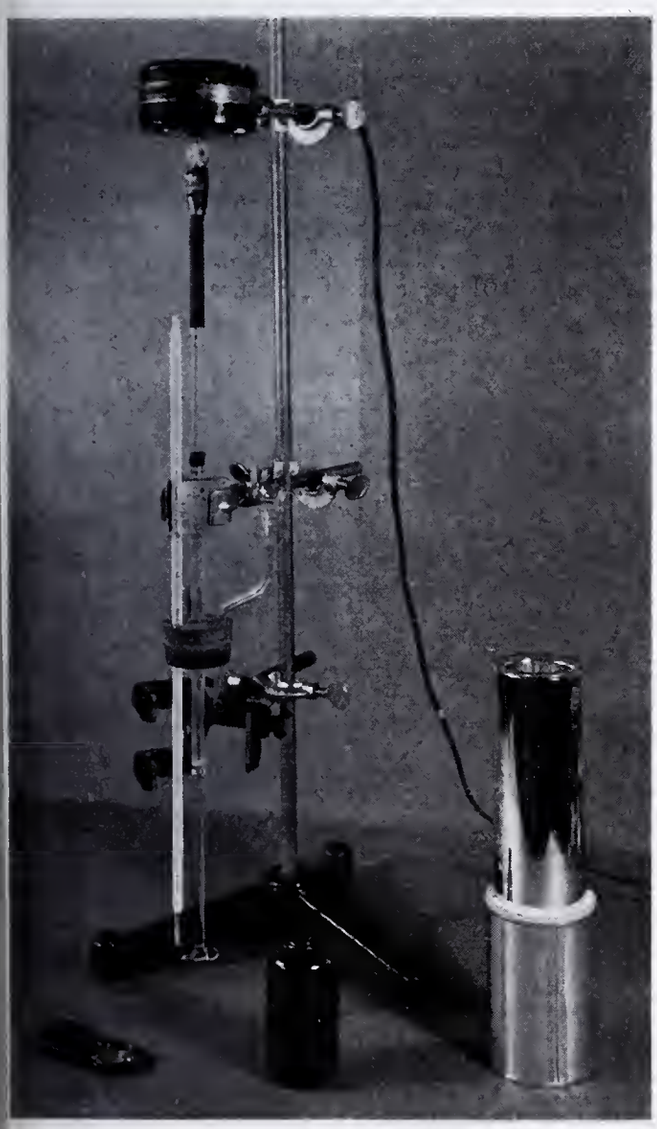

FIGURE 1. EQUIPMENT FOR REACTION-HEAT TEST

It was discovered that the stirrer paddle, which consisted of a 7-mm. glass rod with 19 mm. of one end flattened to a width of 13 mm. and turning at 3000 r. p. m., was not a very efficient stirring device for a two-phase mixture, such as cyclohexane and nitrating acid. This poor mixing tended to lengthen the time of complete reaction between the benzene and the nitrating acid. Therefore, with a 10- to 20-minute period in which to measure the temperature rise, any change in the room temperature and subsequent change in the rate of radiation of heat from the vacuum flask introduced a considerable error.

To overcome these difficulties, it was necessary to construct the apparatus shown in Figures 1 and 2, and to adjust the amount of nitrating acid used, to ensure thorough mixing. The results which follow point out the distinct advantages of this redesigned reaction heat apparatus.

REAGENTS. Nitrating Acid. Mix 2 volumes of 69 to 70 per cent nitric acid (density 1.41) with 1 volume of 95.5 per cent sulfuric acid (density 1.82).

Cyclohexane, density at 20° C. 0.7781, freezing point 6.0° C.

Benzene, density at 20° C. 0.8790, freezing point 5.5° C.

General Procedure

The procedure found most satisfactory for routine work and used, with slight modifications, in the experiments described in this paper, is as follows:

Weigh 50 grams of sample into a 120-ml. (4-ounce) tared bottle (small neck, plastic top) and place this and the bottle of nitrating acid in a constant-temperature bath at 68° F. Pipet 50 ml. of nitrating acid in the vacuum flask and place the flask on the

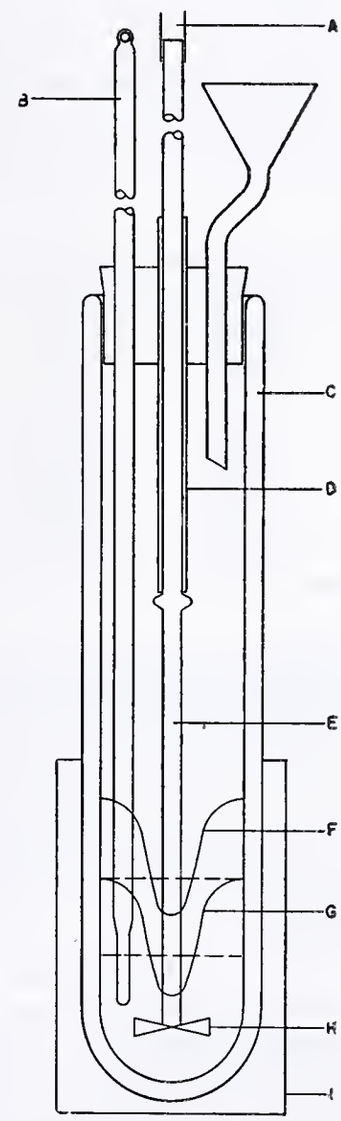

FIGURE 2. ASSEMBLY OF REACTION-HEAT APPARATUS

A. Motor stirrer, 1600 r. p. m.
B. Thermometer, Taylor No. 21,520, length 18 inches, range 45° to 100° F.
C. Vacuum flask, 2 × 10 inch, silvered
D. Glass bearing
E. Glass stir shaft
F. Level of mixture while stirring
G. Level of acid while stirring
H. Glass propeller, 1 × 0.25 inch, approximately 40° pitch
I. Felt-lined metal container

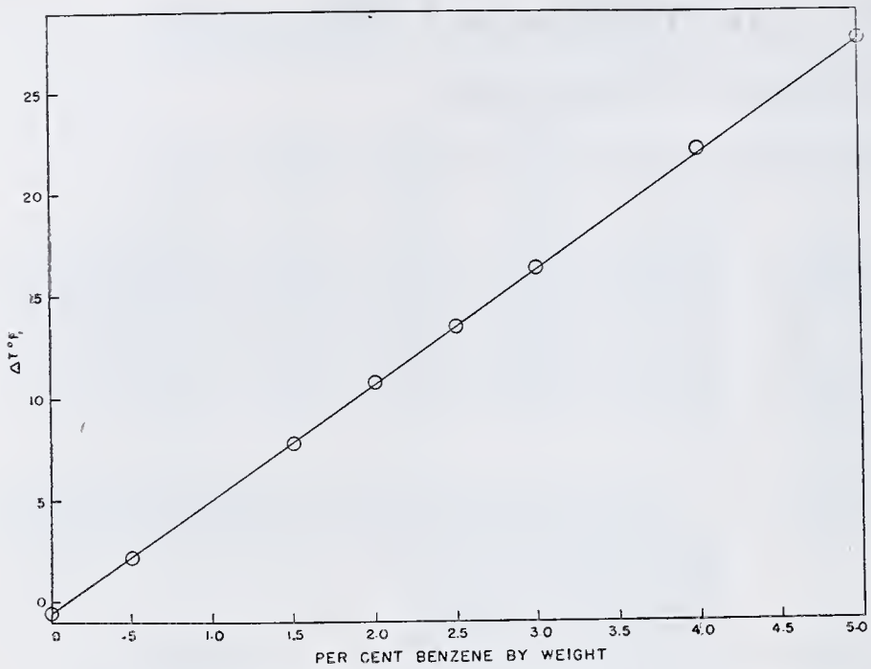

FIGURE 3. REACTION HEAT DERIVED FROM KNOWN MIXTURES OF BENZENE AND CYCLOHEXANE

ΔT. Difference between initial temperature and temperature after 3 minutes of stirring reaction mixture
Nitrating acid, same as No. 2, Table II

TABLE I. REACTION-HEAT TEST DATA (FIGURE 3)

Weight Percentage of Benzene	Initial Temperature ° F.	ΔT ° F.	Room Temperature ° F.
0.0	69.90	−0.60	74
0.5	71.01	2.20	77
1.5	71.25	7.80	76
2.0	70.32	10.80	76
2.5	70.72	13.50	76
3.0	70.75	16.40	76
4.0	71.22	22.20	76
5.0	71.10	27.60	76

stirring device as shown in Figure 2. After 3 minutes of standing, start the motor, and after approximately 1 minute record the "initial" temperature which will be about 70° F. Stop the motor. Pour the sample from the 4-ounce bottle into the vacuum flask and start the motor. Take readings after 1, 2, 3, and 5 minutes of stirring. The difference between the temperature at 3 minutes and the initial temperature is a measure of the benzene content of the sample which is determined from Figure 3.

Discussion

EFFECT OF STIRRING PERIOD. The nitration is complete after 2 minutes of stirring, as shown in Figure 4. Any slope to the curve after the 2-minute period is a result of radiation from the vacuum flask, since the initial temperature of 70° F. is below the room temperature, which is 74° to 76° F.

EFFECT OF IMPROPER MIXING. The most important point in this discussion is the efficiency in the mixing of the reacting substances. It was found that any change in the design of the apparatus, the quantity of reacting substances, or the speed of stirring, which tended to lessen the mixing effect, greatly increased the reaction time and lessened the precision of the results. On the other hand, any change which increased the mixing effect beyond that of the apparatus described in this paper, made almost no change in the results, except to decrease slightly the time of complete reaction. In fact, results were very satisfactory when 100 ml. of nitrating acid and 50 grams of sample were used in a larger vacuum flask (6.25 cm. 2.5 inches in inside diameter) than the one described in this paper.

EFFECT OF STRENGTH OF NITRATING ACID. The effect of acid concentration was tested with acids of the composition shown in Table II. Figure 5 shows that No. 2 acid mixture is very satisfactory. It also has the advantage of being the one most easily prepared from ordinary laboratory C. P. acids and has been chosen therefore for use in the general procedure. A slight variation in the acid strength will not cause any appreciable error in the results. Figure 6 illustrates the variation in ΔT with the time of reaction for a 2 per cent benzene in cyclohexane mixture when nitrating acids of different strengths are used. A similar variation was found to hold true in 0.65, 3.0, and 4.0 per cent benzene-cyclohexane mixtures.

EFFECT OF CERTAIN IMPURITIES IN THE SAMPLE. It was found necessary to determine benzene in cyclohexane which contained a small amount of acetone and methyl alcohol. The reaction between these impurities and the nitrating acid caused a considerable rise in temperature. This difficulty was overcome by extracting the sample with water, and drying with calcium chloride. Any appreciable amount of water in the sample would no doubt cause a rise in temperature. However, a mixture of cyclohexane and benzene contains only 0.01 per cent of moisture when saturated at room temperature.

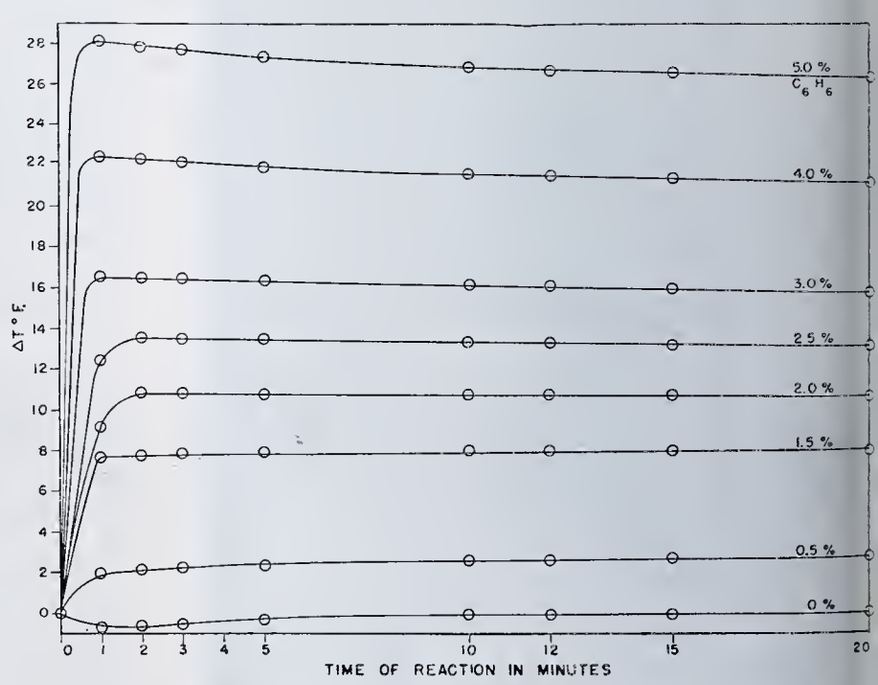

FIGURE 4. VARIATION IN ΔT WITH TIME OF REACTION FOR VARIOUS MIXTURES OF BENZENE IN CYCLOHEXANE

Initial temperature, approximately 70° F.
Nitrating acid, same as No. 2, Table II
Stirring motor was stopped after first 10-minute period

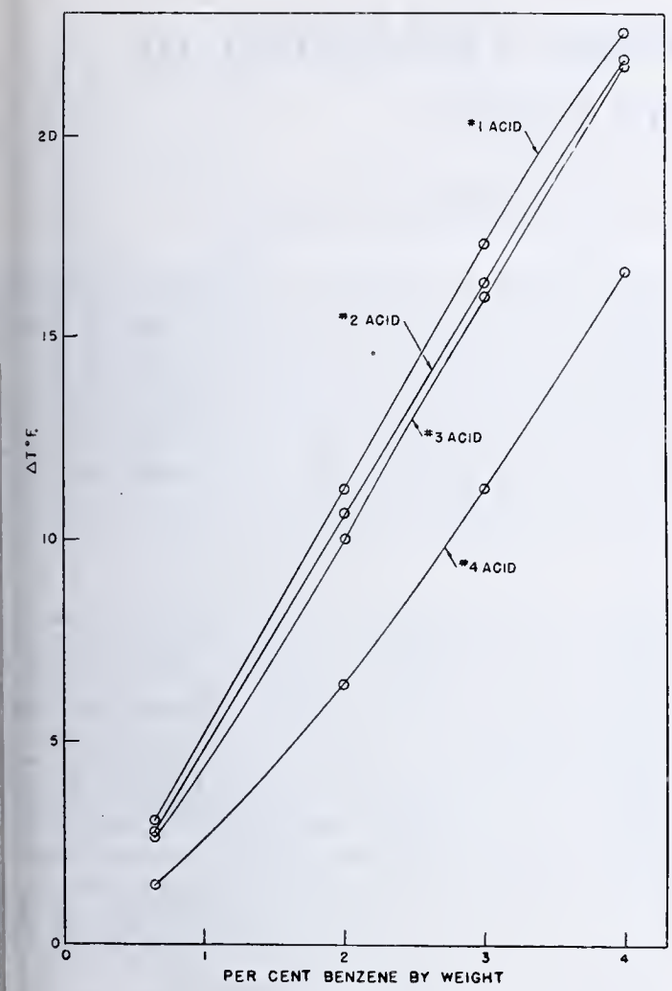

FIGURE 5. REACTION-HEAT DATA FOR KNOWN MIXTURES OF BENZENE IN CYCLOHEXANE USING ACIDS FROM TABLE II

ΔT. Difference between initial temperature and temperature after 3 minutes of stirring reaction mixture

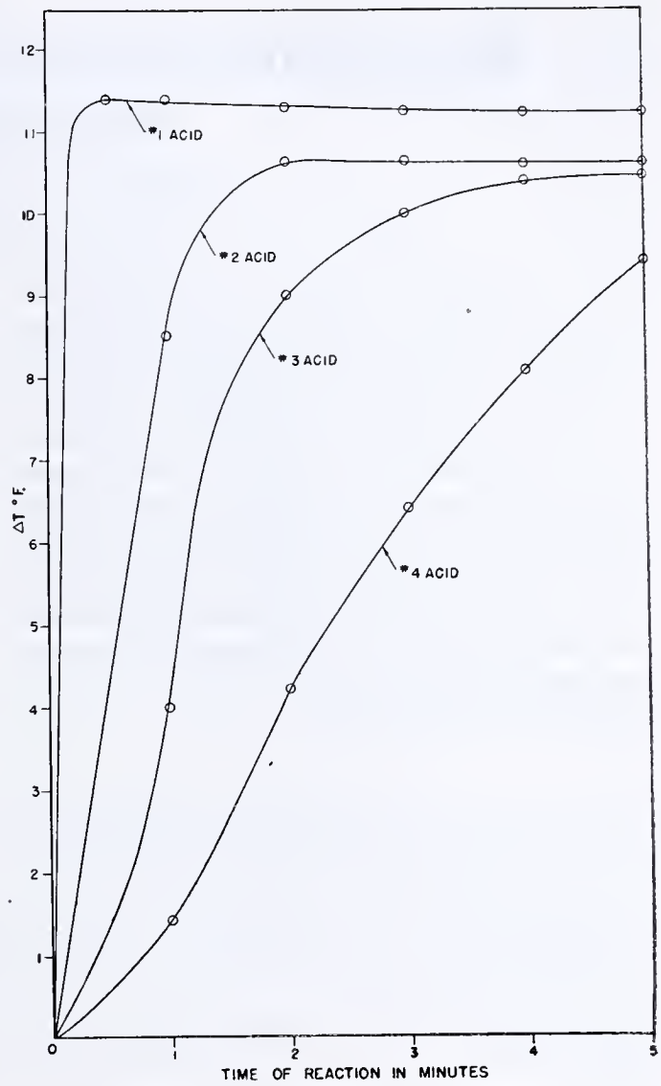

FIGURE 6. VARIATION IN ΔT WITH TIME OF REACTION FOR FOUR STRENGTHS OF NITRATING ACID

Results

The accuracy of the results obtained by the described method is indicated in Table III. These results were obtained on knowns made from mixtures of cyclohexane from various sources, and benzene. Different batches of nitrating acid were used in the course of the experimentation. In each case the acids were made up to approximately the same strength in accordance with the method outlined under reagents.

TABLE II. COMPOSITION OF NITRATING ACIDS (FIGURES 5 AND 6)

Acid No.	HNO_3 %	HNO_2 %	H_2SO_4 %	H_2O %
1	42.94	0.16	38.35	18.55
2	41.32	0.01	38.06	20.61
3	40.58	0.01	37.28	22.13
4	39.77	0.01	36.54	23.68

TABLE III. ANALYSIS OF KNOWNS

ΔT, 3-Minute minus Initial Temperature ° F.	Benzene Present	Benzene Found	Difference
	Weight percentage		
7.82	1.50	1.50	0.00
7.75	1.50	1.47	−0.03
10.63	2.00	2.00	0.00
10.87	2.00	2.04	+0.04
10.48	2.00	1.97	−0.03
10.40	2.00	1.95	−0.05
10.82	2.00	2.02	+0.02
10.98	2.00	2.05	+0.05
10.79	2.00	2.02	+0.02
16.30	3.00	3.00	0.00
16.35	3.00	3.00	0.00
21.78	4.00	3.95	−0.05
22.08	4.00	4.02	+0.02

Summary

The method described in this paper was found to be very workable and rapid, giving a high degree of precision and accuracy. A similar method has been developed to determine benzene in ethylene dichloride and in propylene dichloride, following the same general procedure and using the same strength of acid. However, it was necessary to draw a separate curve of ΔT against per cent benzene for each solvent, as the heat of the nitration reaction in the presence of different types of solvents was not the same.

Acknowledgment

The authors are indebted to F. J. Hopkinson of the Industrial Laboratory for many helpful suggestions and the equipment used in this work, and to S. C. Pool and the staff of the Chemical Plant Laboratory of the Eastman Kodak Company for the analyses of the nitrating acids.

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Determination of Surface Oxidation of Bituminous Coal

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A number of methods for determining the extent of surface oxidation of bituminous coals were examined qualitatively, and two, carboxyl group determination and thermal decomposition of surface complex, were examined in detail. The carboxyl group determination, though rather insensitive, can be used to determine the extent of surface oxidation, but examination of the gaseous products obtained by thermal decomposition of oxidized coals was a more sensitive procedure, particularly in the case of high-rank coals.

IT HAS long been recognized that the oxidation of coal, such as may take place during storage, has a very marked effect on its properties during subsequent carbonization.

Jamieson and Skilling (*9*) used actual carbonization of stored coals to study these effects. Schmidt, Elder, and Davis (*19*) made carbonization tests on mildly oxidized Pittsburgh coal, measuring the relation between the yields and characteristics of the carbonization products, and the oxygen fixed on the coal in the oxidation process. The main effects found were the increased yield and hardness of the coke, the decreased yield of tar, and the increased amount of carbon dioxide in the gas as the oxidation proceeded. They did not carry the oxidation so far as to destroy completely the coking properties. Coking tests on oxidized coals have also been made by Reilly and co-workers (*1, 4, 5*), Michaelis (*13*), Kucherenko (*11*), Purdon (*16*), and Rees (*17*).

That weathered coals have properties similar to humic acids was suggested by Stadnikov, Suiskov, and Ushakova (*20*) and later by Voitova (*26*). Syskov (*22*) and Syskov and Ushakova (*23*) used the absorbing power of oxidized coal for barium hydroxide for evaluation of the extent of the oxidation. Similar measurements with iodine, oxalic acid, and methylene blue gave negative results. Another group of investigators, Karavaev, Rapoport, and Kholler (*10*), compared oxidized coal with brown coal and found these common properties: presence of humic acids, low carbon and high oxygen content, low heat value, excessive hygroscopicity, high content of volatile substances, high content of carbon dioxide in the primary gas, and high reactivity toward nitric acid. In characterizing the "age" of coals by ferric chloride and methylene blue adsorption, Pentegov and Nyankovskiĭ (*14*) found that the coals had to be free from oxidation for a successful application of the test. This loss of reducing power of coal for ferric chloride was investigated as a measure of surface oxidation of coal by Vologdin and Kamendrovskaya (*28*) and by Grigor'ev (*7*). Ferric oxide was also used (*27*). Surface oxidation also brings about the formation of water-soluble materials which will reduce potassium permanganate, as reported by Peters and Cremer (*15*), and also by Bunte, Brueckner, and Bender (*3*). Yohe (*30*) has shown that virgin coal surfaces, after exposure to air, exert a definite oxidizing effect on titanous chloride.

An oxidized coal surface shows a reduced reaction rate as the process proceeds. Bunte and Brueckner (*2*) measured the decrease in air pressure in a closed system and analyzed the remaining gas. Stansfield, Lang, and Gilbart (*21*) measured the amount of oxygen absorbed between the 24th and 96th hours of exposure to oxygen while the sample was being ground in a ball mill. Studies of rates of oxygen absorption have been made by Winmill (*29*) and by Graham (*6, 24*). Schmidt and Elder (*18*) studied the effects of rank, extent of oxidation, temperature, and oxygen partial pressure on the rate. The rate of oxidation by chromic acid has been applied to commercially roasted samples by Howard and Schulz (*8*), who found a decrease in this rate as the roasting proceeded.

Lefebvre and Faivre (*12*) prepared oxidized coal samples so that the amount of oxygen fixed could be calculated, then they heated these to 350° C. and found that most of the fixed oxygen was evolved as carbon monoxide, carbon dioxide, and water. Karavaev *et al.* (*10*) and Schmidt, Elder, and Davis (*19*) reported an increase in the carbon dioxide in the gas during carbonization; the latter workers also reported a lowering of the total gas yield, as did Bunte and Brueckner (*2*).

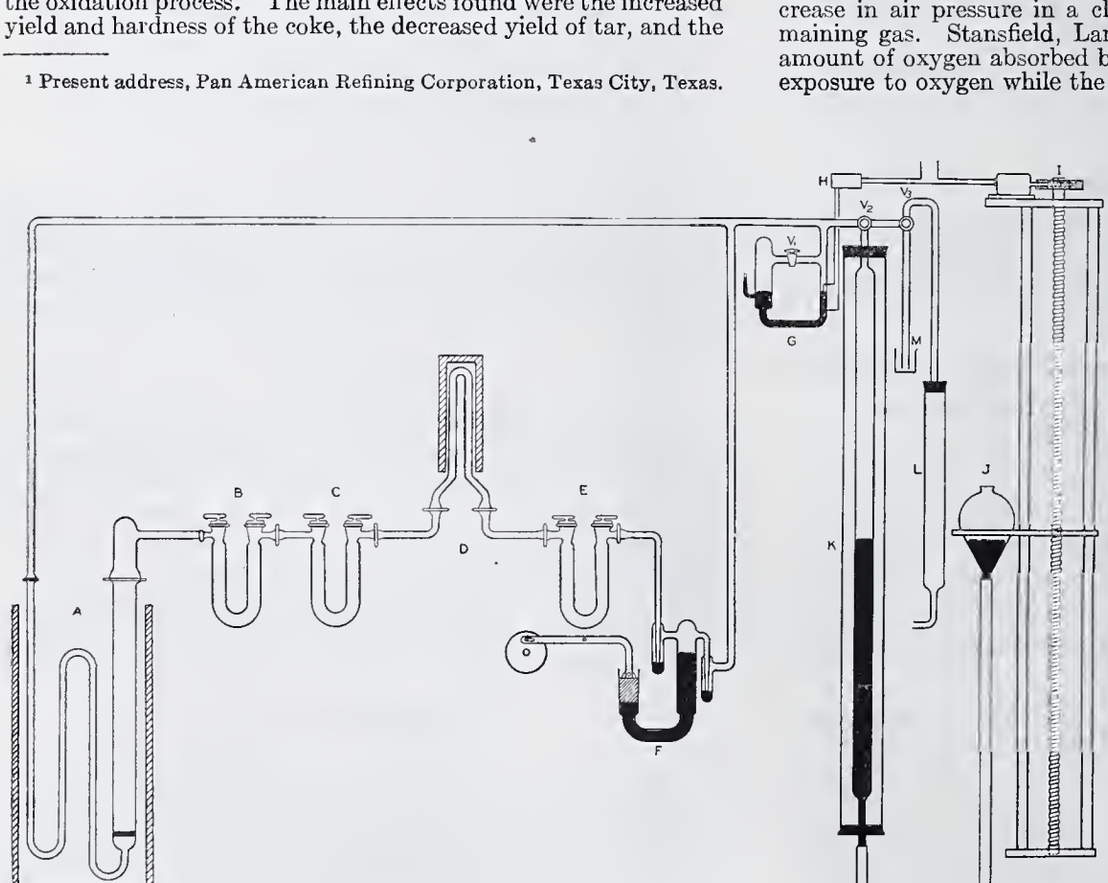

FIGURE 1. APPARATUS FOR OXIDATION OF COAL

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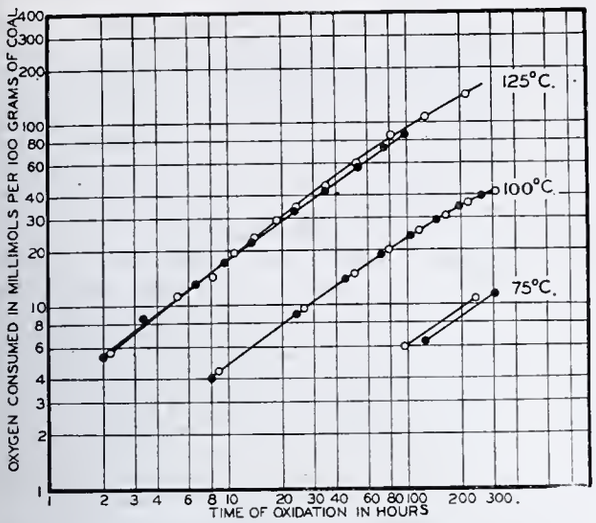

FIGURE 2. RATE OF OXIDATION OF POCAHONTAS COAL

None of these methods was developed to the stage of cceptable laboratory tests for determination of the extent f oxidation of coal. With the object of development of a uantitative method for determination of surface oxidation f coal, preliminary examination was made of a number of he properties of oxidized coal surfaces, using for this purpose sample of Illinois No. 6 coal which had been oxidized by xposure to air in a drying oven at 105° C. for several weeks. Comparisons for both the oxidized coal and an unoxidized ample were made of: (1) sorption of electrolytes, (2) pH of queous suspensions, (3) sorption of basic organic compounds, (4) carboxyl group determination, (5) oxidation-eduction potentials, (6) rates of oxidation, (7) rates of drying, and (8) nature of the thermal decomposition products of he surface complex. Even with this highly oxidized sample f coal, only the carboxyl group determination and nature of he thermal decomposition products of the surface complex ave evidence of being sufficiently sensitive to be of value. Both methods were, therefore, studied further and the results btained are described below. As has also been reported by ther workers, the authors noted the great difference in ease f wetting by water of the fresh and oxidized coal surfaces, he latter being much more readily wet, and it is possible hat some sensitive method based on this phenomenon could e developed. Small but significant differences in the rate f loss of water from fresh and oxidized coal surfaces in an nert gas stream were observed.

Preparation of Samples of Oxidized Coals

Oxidized samples of three typical bituminous coals—Illinois No. 6, Pittsburgh, and Pocahontas No. 3—were prepared in the pparatus shown in Figure 1. A sample of −200-mesh coal, reviously dried in vacuum at 75° C. to 110° C., depending upon the emperature of the run, weighing 50 to 60 grams, was placed in he vertical tube, A, which had a fritted-glass disk sealed in near he bottom to support the coal, and was immersed in a bath held t the desired temperature. Oxygen was circulated up through he coal sample by the mercury piston pump, F, and returned to he pump through absorption tubes B and C, carbon monoxide ombustion tube D, and absorption tube E. Additional oxygen s required was supplied from buret K. Tube B was filled with ndicating Drierite, tubes C and E with Ascarite and Drierite. Tube D was filled with powdered iodine pentoxide mixed with round glass to prevent caking and was kept at 125° C. by the electric heater. Through the manostat arrangement oxygen was upplied to the system as required. The pressure drop resulting rom consumption of oxygen caused the mercury in the manometer, G, to actuate a relay, H, controlling the motor driving the crew elevator, I, which determined the height of the leveling ulb, J, causing oxygen to flow into the circulating system until he pressure was restored and the contacts on G opened again. A 00-ml. water-jacketed buret was employed in all runs except on llinois coal, where at 125° C. one of 1000-ml. capacity was required. To fill the buret, tank oxygen was brought in through the Ascarite tube, L, through stopcocks V_2 and V_3. For this purpose the holder of the leveling bulb, J, could be disengaged from the screw mechanism to lower it and to follow the level in the buret as it filled. V_1 permitted balancing the two sides of the manometer. All runs were at approximately atmospheric pressure.

In this apparatus the total mass of oxygen consumed and the amounts of water, carbon dioxide, and carbon monoxide formed could all be measured. Hence, the amount of oxygen fixed by the coal was readily calculated.

Data on the reaction of the three coals at 75°, 100°, and 125° C. are illustrated by the curves of Figures 2, 3, and 4. The nearly linear log-log relation between oxygen consumed and time has been pointed out by other workers (*16*) in this field. Satisfactory agreement between duplicate runs (open and solid circles) was obtained, except in the case of the Illinois coal at 125° C., where there was difficulty with temperature control, owing to exothermic character of reaction.

The equations for straight lines fitted to the data by least squares were: $\log M = b_1 + b_2 \log t$, where M is in millimoles of oxygen consumed per 100 grams of coal and t is in hours. The values of the constants are given in Table I.

The values of the intercepts, b_1, show very definitely that the reactivities of the coals increase with decrease in rank. The form of the curves and the nearly constant values of the slopes for the various coals indicate hindered reactions and a relatively constant hindrance, irrespective of the reactivity of the coal and the temperature.

Data giving the distribution of the oxygen between the gaseous products and the coal surface are shown in Table II. Although total oxygen consumed varied more than tenfold with different temperatures and reaction times, the per cent distribution among the different products is surprisingly constant.

TABLE I. CONSTANTS IN EQUATIONS FOR REACTION RATES

	75° C.		100° C.		125° C.	
	b_1	b_2	b_1	b_2	b_1	b_2
Pocahontas No. 3	−0.532	0.657	+0.069	0.638	+0.526	0.714
	−0.613	0.671	−0.021	0.683	+0.550	0.711
Pittsburgh	−0.134	0.723	+0.398	0.750	+0.955	0.761
	−0.095	0.713	+0.485	0.672	+0.090	0.695
			+0.409	0.731		
Illinois No. 6	+0.333	0.615	+0.893	0.592	+1.815	0.377
	+0.233	0.655	+0.910	0.651	+1.583	0.447

Determination of Carboxyl Groups

This procedure for determining the acidic groups on the surface of the oxidized coal is essentially the same as that

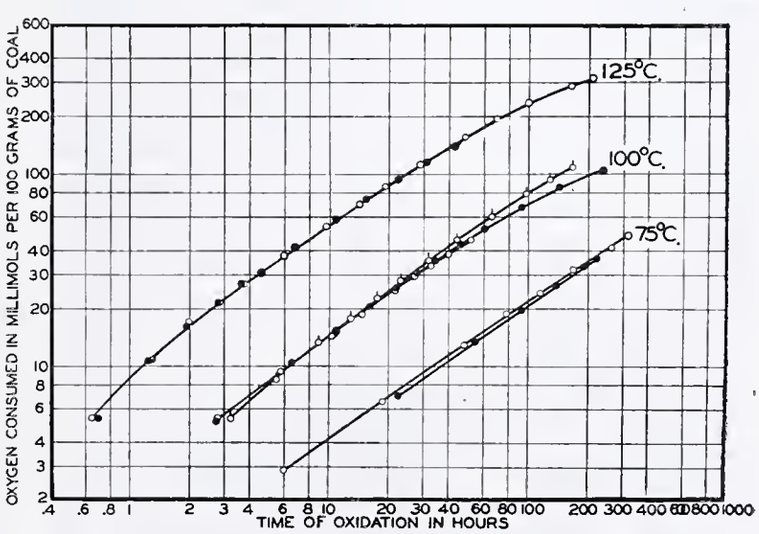

FIGURE 3. RATE OF OXIDATION OF PITTSBURGH COAL

described by Ubaldini and Siniramed (*25*) for determining the equivalent weights of naturally occurring "humic acids". In the Coal Research Laboratory this method has been used in obtaining the equivalent weights of "humic acids" prepared by the oxidation of bituminous coals. The procedure is based on the decomposition of calcium acetate by the acid groups and the determination of the resulting acetic acid by the liberation of carbon dioxide from calcium carbonate. The equations for the reactions are:

$$2RCOOH + Ca(OAc)_2 \longrightarrow (RCOO)_2Ca + 2HOAc$$

$$2HOAc + CaCO_3 \longrightarrow Ca(OAc)_2 + H_2O + CO_2$$

or

$$2RCOOH + CaCO_3 \longrightarrow (RCOO)_2Ca + H_2O + CO_2$$

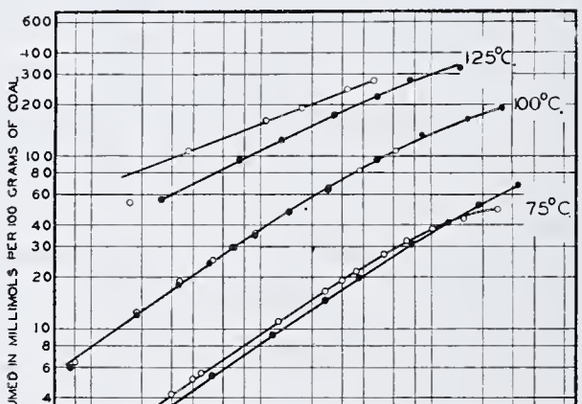

FIGURE 4. RATE OF OXIDATION OF ILLINOIS COAL

Whether the reaction proceeds in one manner or the other, one mole of carbon dioxide is liberated for each two carboxyl groups. The reaction is carried to completion by refluxing the mixture and sweeping out the carbon dioxide formed with nitrogen. The apparatus used is shown in Figure 5.

Before this method was applied to oxidized coals its reliability was tested on a number of authentic samples of organic acids. Succinic, benzoic, *o*-phthalic, *o*-, *m*-, and *p*-hydroxybenzoic, and gallic acids were employed for this purpose. The simple carboxylic acids gave results for equivalent weight which were 1 to 2 per cent high—i. e., less than the theoretical amounts of carbon dioxide were evolved during the reaction. With the hydroxy acids the results were all low by about the same amount and carbon dioxide continued to be evolved slowly if the reaction time was increased. With the hydroxybenzoic acids the equivalent weights calculated on reaction of the carboxyl alone were too low by about 10 per cent when a reaction time of 4 hours was used. With gallic acid the values were almost exactly half that calculated for reaction of the carboxyl. That this excess evolution of carbon dioxide was due to reaction of the phenolic hydroxyls was shown by the fact that the methoxy compounds of these acids gave results agreeing with the theoretical values within 1 per cent. Since in a measurement of surface oxidation of coal it is immaterial what type of acidic group reacts, the procedure appeared suitable for the purpose desired.

The finely ground sample was placed in flask *D*, the weigh being adjusted to evolve a convenient amount of carbon dioxide 1 gram of calcium carbonate and 100 ml. of 3 per cent calciu acetate were then added, the flask was attached to the reflux con denser, *C*, and the mixture was boiled with a microburne Tank nitrogen purified by passage over hot reduced copper wa used as a sweeping gas and passed through the sulfuric aci bubble counter, *A*, and Ascarite tube, *B*, down through the lon tube in the center of the condenser and bubbled through th boiling mixture. The evolved carbon dioxide was swept ou through the condenser, sulfuric acid bubble counter, *E*, and dry ing tube, *F*, filled with Anhydrone, into the weighing tube, *G* containing Ascarite and Anhydrone. The nitrogen passed ou through the guard tube, *H*, filled with soda lime, the trap, *I*, an the bubble counter, *J*, to the atmosphere. Under these condi tions a manipulative blank of 1 to 2 mg. of carbon dioxide wa obtained. This blank could be reduced to 0.2 mg. by a shor preliminary refluxing of the reagents before addition of the sam ple. With slightly oxidized coals difficulty in wetting was ob served; part of the sample floated on the surface and was carrie up the side of the flask out of contact with the bulk of the re agents. In such cases addition of 10 ml. of a 1 per cent solutio of a wetting agent, such as Aerosol or Nekal BX, was found to im prove the reproducibility of the procedure.

With the lightly oxidized samples of coal the amounts c carbon dioxide evolved by this procedure were only a fe milligrams per gram of coal, so that it was necessary to us large samples, 5 to 10 grams, and to weigh the absorptio tube on a semimicrobalance. Weights were recorded to 0. mg.

The results of determinations of the acid groups on th prepared samples of oxidized coals are shown in Table II and Figure 6. The total acidity has been calculated in term of carboxyl, since it seems probable that these groups ar chiefly responsible for the reaction observed. In calcu lating carboxyl groups the amount of carbon dioxid evolved from the unoxidized coal was subtracted from th values for the different oxidized samples. The differenc multiplied by two, since each mole of calcium salt reacts wit two carboxyl groups, yields the carboxyl groups formed i the oxidation. The amount of carbon dioxide evolved fro the unoxidized Pocahontas coal corresponds to the presenc of approximately 0.01 per cent of oxygen as carboxyl grou and the corresponding figures for Pittsburgh and Illinois a 0.10 and 0.13 per cent, respectively. In the case of th Pocahontas coal there was a somewhat smaller evolution c carbon dioxide from the sample which had been oxidized a 75° than from the original unoxidized samples. In Figure carboxyl groups, in millimoles per 100 grams of coal, foun

TABLE II. DISTRIBUTION OF OXYGEN IN OXIDIZED COALS

Temperature	Time	O_2 Used	H_2O	CO_2	CO	Fixe
° C.	Hours	Millimoles per 100 g.	%	%	%	%
		Pocahontas No. 3 Coal				
75	240	10.86	30.85	11.05	1.66	56.4
75	306	11.40	29.20	11.31	1.58	57.9
100	261	39.40	31.60	12.80	2.04	53.5
100	312	40.90	32.16	12.84	1.81	53.1
125	99	86.40	29.85	13.19	2.08	54.8
125	215	143.0	27.62	14.75	2.38	55.2
		Pittsburgh Coal				
75	223	36.49	18.82	14.44	1.70	65.0
75	319	48.38	21.19	9.06	1.59	68.1
100	240	104.8	27.77	12.50	2.10	57.6
100	52	45.7	19.04	10.06	1.53	69.3
125	43	139.4	17.93	15.06	2.01	65.0
125	212	316.0	27.17	18.77	2.47	51.6
		Illinois No. 6 Coal				
75	246.5	49.35	18.27	13.31	2.09	66.3
75	323.0	68.27	20.21	12.15	1.96	65.3
100	260	189.8	33.14	18.07	2.53	46.2
100	62	106.9	24.50	13.84	2.06	59.6
125	46	275.4	34.77	16.22	3.59	45.3
125	148	327.4	29.9	19.16	2.23	48.7

by this method, are plotted as a function of the amount of oxygen known to have been fixed in preparing the sample. It is evident that only a fraction of the total oxygen fixed reacts like a carboxyl group and that this fraction varies greatly with the coal, ranging from a few per cent for the Pocahontas up to 40 per cent for the Illinois. There is, however, for each coal a general linear relation between the oxygen found as carboxyls and the total fixed. The method is obviously less sensitive for high-rank coals. With a Pittsburgh seam coal, samples prepared containing 1.0 per cent by weight of "fixed" oxygen evolve approximately 1 mg. of carbon dioxide per gram of sample.

Thermal Decomposition of the Surface Complex

It is known that the gaseous products obtained from the thermal decomposition of partly oxidized coals contain larger proportions of the oxides of carbon than do those from virgin coals.

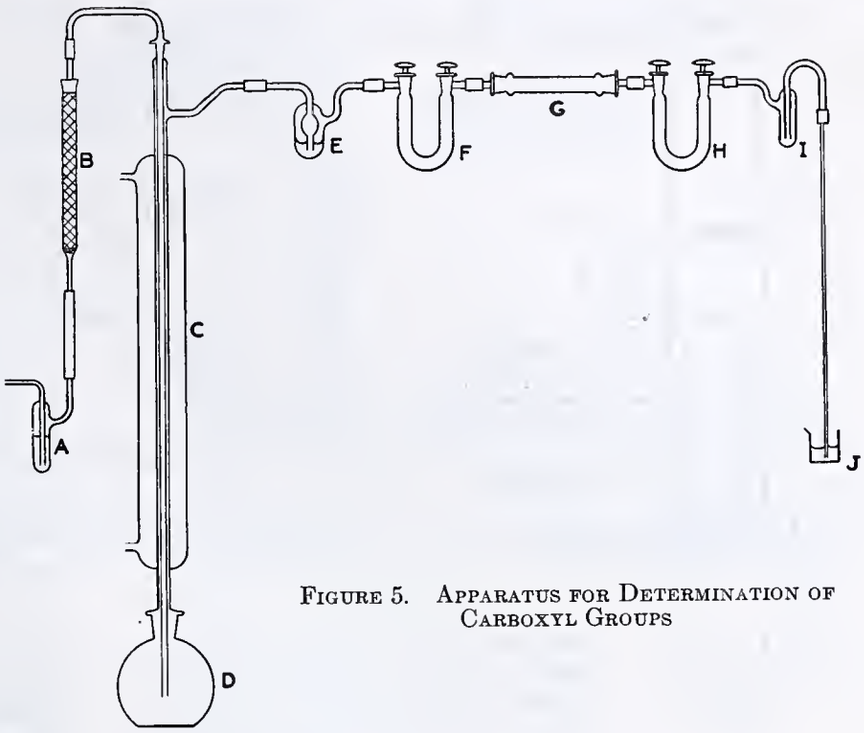

Figure 5. Apparatus for Determination of Carboxyl Groups

Table III. Determination of Acid Groups on Oxidized Coals

Temperature of Oxidation ° C.	Time Hours	O_2 Fixed	CO_2	Carboxyl
		—Millimoles per 100 grams—		
		Pocahontas No. 3 Coal		
..	0	..	0.36	..
75	240	6.13	0.31	..
75	306	6.60	0.29	..
100	261	21.1	0.52	0.32
100	312	21.8	0.61	0.50
125	99	47.4	0.66	0.60
125	215	79.0	0.95	1.18
		Pittsburgh Coal		
..	0	..	3.14	..
75	223	23.75	..	..
75	319	32.98	..	..
100	168	66.1	5.71	5.14
100	240	60.4	5.07	3.86
100	52	31.7	4.43	2.58
125	43	90.6	5.09	3.90
125	212	163.1	7.46	8.64
30	507	..	3.64	1.00
		Illinois No. 6 Coal		
..	0	..	4.28	..
75	246.5	32.73	11.02	13.58
75	323.0	44.83	..	..
100	260	87.8	12.56	16.56
100	62	63.7	8.95	9.34
125	46	125.0	14.98	21.40
125	148	159.6	15.32	22.08

In order to collect the gaseous products quantitatively, approximately 10 grams of the coal sample were placed in the bottom of a small molecular still, such as that shown in Figure 7. The still was evacuated through a small multiple-stage mercury diffusion pump which was in turn backed by a Toepler. The discharge tube of the Toepler delivered the evolved gases into a mercury-filled gas sampling tube. The collected gas was measured and analyzed in an Orsat apparatus and the volumes were calculated to standard conditions. It was found convenient to cover the coal sample with a 200-mesh nickel screen to prevent spurting when gas evolution became rapid. The condenser of the still was kept filled with a dry ice–ethylene chloride mixture to freeze out water and hydrocarbon vapors. Heat was applied to the still by means of an electrically heated aluminum block. Temperatures were controlled to ±5° C. The whole system was thoroughly evacuated at room temperature, 10^{-3} mm. of mercury, raised to 350° in 45 minutes, and maintained at that temperature for 3 hours with continuous evacuation.

The results are shown in Table IV and Figure 8. It is evident that this is a more sensitive procedure for determining surface oxidation of coal, since a much larger fraction of the oxygen appears as a measurable product, in this case oxides of carbon, than was the case in the carboxyl group determination. The particular experimental arrangements used did not permit the determination of water vapor. Modification of the analytical procedure to permit determination of the water evolved might increase the sensitivity considerably, since it seems probable that, as in the oxidation step itself, the larger fraction of the oxygen appearing as volatile products will be as water. Carbon monoxide appeared in all samples in small amounts.

Summary

A number of possible methods for determining the extent of surface oxidation of bituminous coals have been examined qualitatively. These were sorption of electrolytes; pH of aqueous suspensions; sorption of basic organic compounds; carboxyl group determination; oxidation-reduction potential; rate of oxidation; rate of drying; thermal decomposition of the surface complex. Of these, only two—carboxyl group determination and thermal decomposition of surface complex—showed sufficient promise to justify detailed

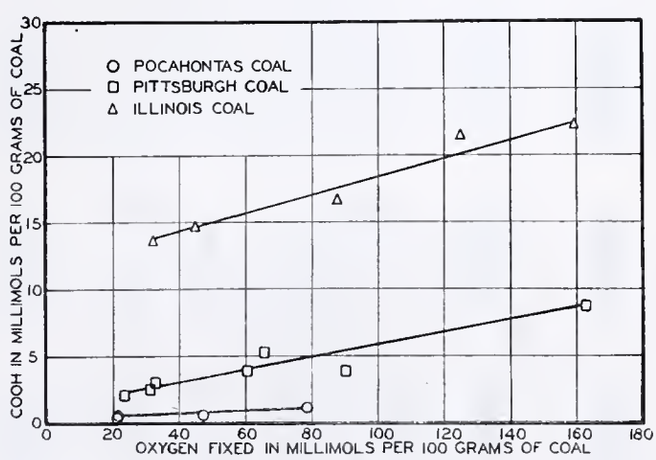

Figure 6. Carboxyl Groups on Coal as a Function of Degree of Oxidation

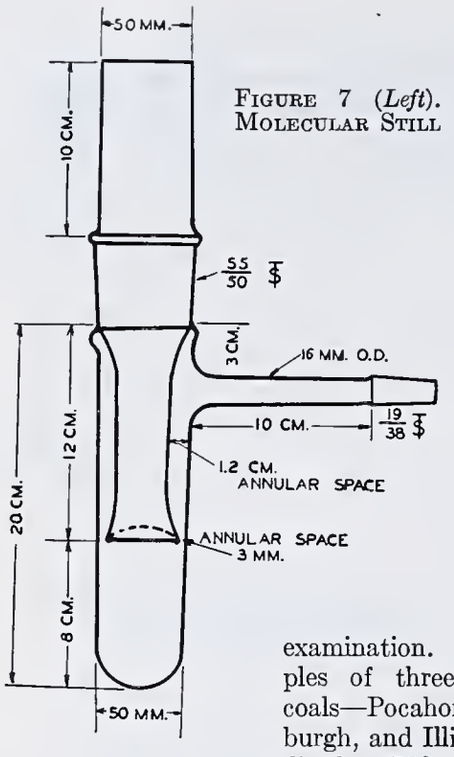

FIGURE 7 (*Left*). MOLECULAR STILL

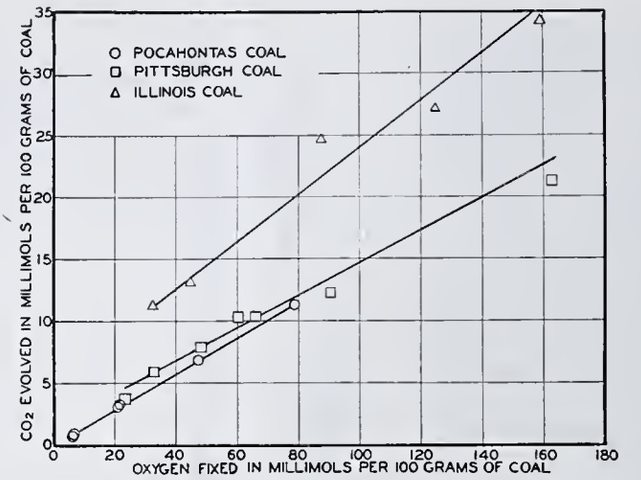

FIGURE 8. CARBON DIOXIDE FROM DECOMPOSITION OF COAL AS A FUNCTION OF DEGREE OF OXIDATION

examination. For this purpose samples of three typical bituminous coals—Pocahontas No. 3, Pittsburgh, and Illinois No. 6—were oxidized at 75°, 100°, and 125° C. for varying periods and under such conditions that the extent of oxidation was definitely known.

Carboxyl group determinations showed that only a fraction of the total oxygen fixed reacts like a carboxyl group, ranging from a few per cent for the Pocahontas coal up to 40 per cent for the Illinois. A general linear relation was found, however, between the oxygen as carboxyls and the total known to be present. Hence, this procedure, though rather insensitive, can be used for the determination of the extent of surface oxidation.

Examination of the gaseous products obtained by thermal decomposition of these oxidized coals in vacuum at 350° C. also showed a linear relation between oxygen fixed and that evolved as oxides of carbon, and since a larger fraction of the fixed oxygen was so evolved than in the above test, this is a more sensitive procedure for the measurement of the extent of surface oxidation, particularly in the case of high-rank coals.

More exact evaluation of the usefulness of these methods depends upon their application to naturally aged coal samples and correlation of the analytical results with carbonization tests.

TABLE IV. CARBON OXIDES EVOLVED IN VACUUM DECOMPOSITION OF OXIDIZED COALS

Temperature of Oxidation	Time	O_2 Fixed	Total Gas	CO_2	CO	CO_2
						Millimoles/ 100 g. less blank
°C.	*Hours*	*Millimoles per 100 grams*				
		Pocahontas No. 3 Coal				
..	0	..	4.90	2.39	0.14	..
75	240	6.13	5.22	3.06	..	0.677
75	306	6.60	5.56	3.29	..	0.907
100	261	21.1	8.84	5.37	0.20	2.99
100	312	21.8	11.61	5.66	0.27	3.27
125	99	47.4	16.68	9.26	..	6.87
125	215	79.0	22.33	13.58	1.28	11.19
		Pittsburgh Coal				
..	0	..	11.93	4.92	0.24	..
75	223	23.73	16.11	8.71	0.13	3.79
75	319	32.98	17.43	10.84	..	5.92
100	168	66.1	27.17	15.24	0.77	10.32
100	240	60.4	25.57	15.19	..	10.27
125	43	90.6	27.69	17.14	0.84	12.22
125	212	163.1	40.30	26.16	2.09	21.24
		Illinois No. 6 Coal				
..	0	..	24.75	13.72	0.47	..
75	247	32.73	41.20	24.92	0.89	11.20
75	323	44.83	45.68	26.78	3.79	13.06
100	260	87.8	61.00	38.43	1.90	24.71
100	62	63.7	42.72	24.96	0.06	11.24
125	46	125.0	65.70	40.88	4.96	27.16
125	148	159.6	75.73	47.90	6.12	34.18

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Determination of Halogen in Organic Compounds

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A method is described for the determination of chlorine, bromine, and iodine in organic compounds. The sample is decomposed by vaporizing it into a stream of combustible gas which is then burned at a jet. The products of combustion are collected in a suitable absorption medium, and the resulting alkali halide is determined by a standard method. This procedure has been employed with thirty-six different compounds, covering a wide range of physical properties from low-boiling liquids (gases) to high-melting solids, some of which contained more than one halogen. By the substitution of silver, lead, or copper for glass in certain parts of the apparatus, the method should be equally satisfactory for the analysis of organic fluoro- compounds. The suggested procedure is economical of time and equipment, and compares favorably with other methods in accuracy and precision.

THE literature of organic halogen determination has been surveyed in some detail recently (*3*), and need not be eviewed here. This paper presents a method of decomposing he sample and recovering the halogen in a form suitable for letermination by any well-known standard method. The rincipal advantages offered over others in common use are he short time required for an analysis, the simplicity of the pparatus without sacrifice of accuracy and precision, and he ease with which such stable compounds as the chloroluoromethanes are decomposed.

The nearest approach to the method described below is hat of Cadenbach (*1*) for the determination of fluorine. Cadenbach vaporized or decomposed the sample in a stream f hydrogen in a silver tube, and burned the gas mixture t a jet, collecting the products through a silver funnel and ube in a standard solution of alkali contained in a gasvashing bottle.

A modification of the lamp method of determining sulfur n gasoline has been applied to the determination of small mounts of chlorine in gasoline (*6*). It is assumed that the hlorine emerges from the combustion process in the form of ydrochloric acid, and that it can be determined by absorpion in a standard solution of alkali. This assumption may, erhaps, be valid when the chlorine content of the sample is ery low, but if more than a trace of halogen is present, some f it is oxidized beyond the halide stage. The free halogen nay be readily detected in the products of combustion. n order to ensure the quantitative recovery of the halogen s halide ion, the products of combustion must be collected n an alkaline solution containing a reducing agent which will not interfere in the subsequent determination of the halogen.

The work of Hass and others (*4*) on the chlorination of methane required the determination of methyl chloride in a mixture with methane. They mixed the sample with an excess of air and passed it through a clay combustion tube at 1000° C., collected the products of combustion in wash bottles containing a solution of sodium carbonate and sodium arsenite, and titrated the chloride by the Mohr method. Their procedure was adapted from one described earlier by Martinek and Marti (*5*).

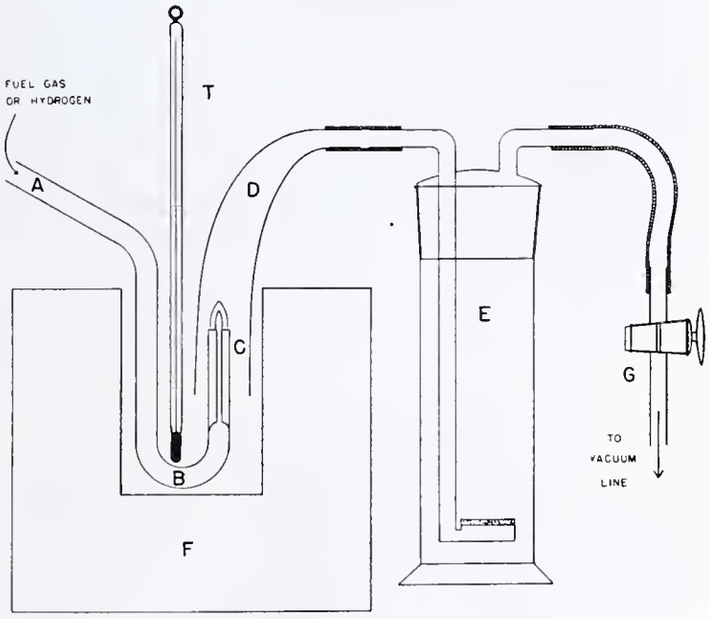

FIGURE 1. APPARATUS FOR SOLIDS AND HIGH-BOILING LIQUIDS

In the method of analysis proposed in this report, the sample is decomposed by burning it in a stream of halogen-free combustible gas at a small jet under a chimney through which the products of combustion are carried away in a stream of air drawn through a gas-washing bottle containing an absorption solution of sodium carbonate or hydroxide and sodium arsenite. After the combustion of the sample, the halide is determined in the absorption solution by a modified Volhard procedure.

Reagents and Solutions Required

The solution used in the gas-washing bottle to absorb the halogen was approximately 0.2 *N* with respect to sodium carbonate or hydroxide, and 0.1 *N* with respect to sodium arsenite. The sodium arsenite is readily obtained by dissolving the requisite amount of arsenic trioxide in a little sodium hydroxide. The final solution should be tested to be sure that it is free of halide.

The inorganic halide ion in the absorption solution may be determined by any suitable method, but the Caldwell and Moyer modification (*2*) of the volumetric procedure of Volhard was chosen for its convenience in making a large number of determinations. A 0.1 *N* solution of silver nitrate and a 0.05 *N* solu-

tion of potassium thiocyanate were prepared and standardized against pure sodium chloride and against constant-boiling hydrochloric acid. Other reagents needed are halogen-free concentrated nitric acid, nitrobenzene, and ferric sulfate.

Description of Apparatus

Two forms of apparatus are required, one for solid and high-boiling (above 100° C.) liquid samples, and the other for low-boiling liquids and condensable gases.

Figure 1 shows the apparatus used for solids and high-boiling liquids. *ABC* is a U-tube made of 9-mm. glass tubing on the *A* side, and of 1-mm. capillary on the *C* side. It may be suspended by a wire loop from the stirrup hook of the balance, thus serving as the vessel for weighing the sample. Solid samples are introduced at *A* and shaken down to *B*. Liquids are best introduced at *C* by means of a medicine dropper drawn out to a fine capillary. If both legs of the U-tube are capillary, liquids boiling as low as 80° C. may be handled satisfactorily, but as a rule it is better to use this form of apparatus only with the higher-boiling substances. A large sample may be burned by increasing the size of the *B* portion of *ABC*.

D is an ordinary glass adapter commonly found in any laboratory, serving as a chimney in which the flame burns in a stream of air drawn through the absorber, *E*. If *E* is provided with a fritted-glass dispersion disk, a single absorber is sufficient. *G* is a stopcock to control the rate of flow of the air stream. *F* is a small crucible furnace used to melt and vaporize the sample. The heat of this furnace is controlled by means of a variable transformer. A thermometer, *T*, assists in regulating the temperature in the furnace. It is necessary that the *C* side of the U-tube be entirely within the furnace, in order to avoid condensation of the sample in the capillary. The whole apparatus, except the furnace, is mounted on one supporting ring stand, so that the assembly may be handled as a unit and lifted into or out of the furnace without interrupting combustion. The dimensions of the various parts are not very critical, and are determined largely by the materials at hand. It is suggested, however, that the absorber be of 200- to 250-ml. capacity, and the dispersion disk of medium porosity.

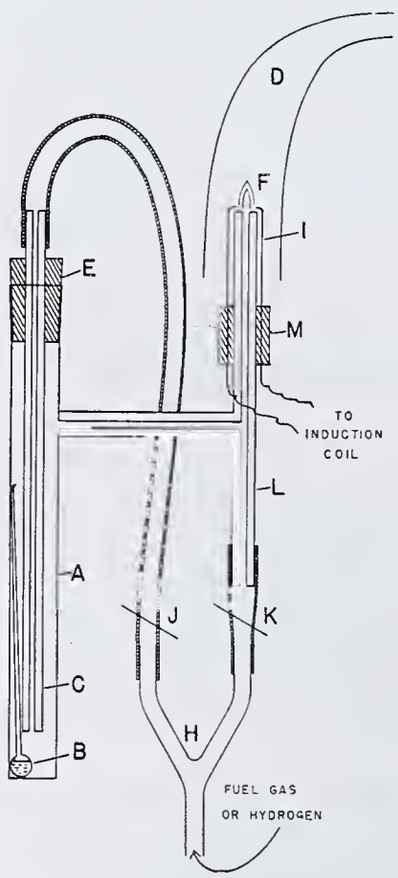

Figure 2. Apparatus for Low-Boiling Liquids

Figure 2 shows the apparatus used for low-boiling liquid samples. The weighed sample contained in the glass bulb, *B*, evaporates into *A* when the stem of the bulb is broken, and the vapor is swept out by a stream of combustible gas entering through the tube, *C*, and is burned at *F* under the chimney, *D*. Some of the gas which burns at *F* is introduced through the side arm, *L*, in order to keep the flame burning when the mixture coming from *A* may be too rich to burn by itself. Screw clamps on the rubber tubing at *J* and *K* control the flow of gas. A slight file scratch is made on the stem of the sample bulb before it is introduced into *A*, and the stem is broken off by manipulation of tube *C* in the flexible rubber stopper, *E*. In the case of highly volatile samples, *A* must be cooled before the sample bulb is opened; and the less volatile samples must eventually be warmed gently to hasten evaporation. This may be accomplished by means of a suitable liquid bath surrounding tube *A*. The spark igniter, *I*, is not absolutely essential but it is very convenient and often saves a determination when the flame happens to go out when the sample bulb is opened. It consists of two pieces of Nichrome wire mounted through a piece of rubber stopper, *M*, and connected to an induction coil. The absorber and chimney are the same as in Figure 1.

The preparation of sample bulbs requires no great glass-blowing skill. A fine capillary, 0.5 to 1 mm. in diameter and 25 cm. in length, is drawn from Pyrex tubing in the usual manner, a thin bulb 6 or 7 mm. in diameter is blown on one end, and the stem is bent in the middle to an angle of 45°. After weighing the empty bulb, the open end of the capillary stem is put into the liquid to be sampled and the bulb is immersed in a cold bath. For some substances an ice-water bath is cold enough, but others will require a bath such as acetone and dry ice. A little liquid is drawn into the chilled bulb, but not enough to fill it. It is then withdrawn from the bath and warmed a little with the fingers, thus expelling more or less of the air. A second immersion in the cold bath will usually draw in a sufficient sample. In no case should the bulb be completely filled; it will burst on warming to room temperature unless there is some free space to allow for expansion of the liquid. When the proper amount of sample has been drawn into the bulb, the steam is sealed off at the bend while the bulb is still in the cold bath. The sealed off portion of the stem is passed through a flame to remove any liquid remaining in it, and the filled bulb and stem are weighed together after they have come to room temperature.

Details of Analytical Procedure

Solid Samples. Weigh the clean and dry U-tube (*ABC*, Figure 1) suspended from the stirrup hook of the balance by means of a wire loop. Introduce the appropriate amount of sample (usually 0.1 to 0.2 gram) through *A*, and shake it down to *B*. Weigh the tube and contents.

Assemble the apparatus as shown in Figure 1. Absorber *E* should contain approximately 50 ml. of the carbonate-arsenite solution, and cock *G* should have been previously adjusted to allow just enough air through the system to support the flame. Turn on the gas enough to give a flame 1 cm. high, ignite it at *C*, and at once put the chimney over the flame. Turn on the current in heater *F* and adjust it so that the temperature rises slowly toward the sublimation point or boiling point of the sample. The volatilization characteristics of various substances differ greatly, but experience soon teaches the operator the proper handling in each case. The character of the flame serves as a useful and convenient guide to the proper heat treatment of the sample. Some organic halogen compounds impart a fairly bright blue or green color to the nearly colorless flame of the city gas, while others make it brilliantly luminous with a white or yellow light, and if the vaporization rate is too great the flame usually becomes smoky and soot may be deposited in the chimney.

Soon after the sample has disappeared and the color of the flame indicates that it is all burned, turn off the gas, disassemble the apparatus, remove the chimney from the absorber, and rinse carefully into the absorption flask the inlet tube and dispersion disk and the inside of the cap. To the solution in the flask, add either from a buret or by means of a transfer pipet a slight excess of the standard 0.1 *N* silver nitrate solution. A sufficient excess of silver nitrate is indicated by the appearance of a yellow-brown precipitate of silver arsenite in the alkaline solution. Add 2 ml. of concentrated nitric acid, 0.5 gram of ferric sulfate (powdered solid), and 3 ml. of nitrobenzene for each 10 ml. of silver nitrate used. Stir the mixture vigorously until the precipitate has coagulated, and then titrate the excess silver with the standard 0.05 *N* thiocyanate solution. The author prefers to add both standard solutions from burets and to cross the end point several times in each direction.

High-Boiling Liquid Samples. If the substance boils above 100° C., proceed as described above for solid substances, but introduce the sample at *C* by means of a capillary medicine dropper.

Low-Boiling Liquid Samples. If the substance to be analyzed boils below 80° to 100° C., it probably cannot be weighed and burned in the apparatus shown in Figure 1 without losing

some of the sample. It must, therefore, be sealed in a small glass bulb for weighing, and burned in the apparatus shown in Figure 2. The sample bulbs are made and filled as previously directed.

Make a small file scratch on the stem of the sealed bulb containing the weighed sample, 2 or 3 cm. from the bulb, and carefully insert the bulb into tube *A* (Figure 2). Replace tube *C*, taking care not to break the sample bulb or its stem. Open the screw clamp, *J*, until *A* is filled with the fuel gas or hydrogen. Then close *J* and open *K*, ignite the gas at *F*, and adjust *K* to give a flame 1 cm. high. The sample must then be cooled, if necessary, to bring it somewhat below its normal boiling point in order to prevent too rapid evaporation when the bulb is opened. This may be easily accomplished by immersing *A* in the same cooling bath that was used in sealing the sample bulb. If the boiling point of the sample is considerably above room temperature, no cooling will be necessary. Now assemble the absorber as described above, place the chimney over the flame, and break the stem of the sample bulb by manipulating *C* in the flexible rubber stopper, *E*. Open *J* a little and partially close *K* to maintain the flame at a height of 1 cm.

If the sample boils below room temperature, it will evaporate satisfactorily without further attention as *A* gradually warms up to room temperature. Less volatile substances will require the application of some heat to hasten the evaporation. This is best accomplished by means of a water bath around *A*, which is heated slowly toward, and eventually above, the boiling point of the sample. After the sample has all evaporated, close *K* and open *J* a little more, and allow the fuel gas to sweep through *A* for a few minutes after the flame color indicates that the sample is all burned. Determine the halide in the absorption solution as described above for solid samples.

Discussion of Method and Results

The accuracy and precision of the procedures may be judged by the results listed in Table I. Some of these compounds were purified as indicated in the table, and in each case the halogen content was checked by the peroxide bomb method of decomposing the sample. The average results by the proposed method are in substantial agreement with those obtained by the older method, and the average deviation from the average value is not much over 0.1 per cent of the average value. This is the order of magnitude of the precision of ordinary analytical work. Further refinements in the technique would undoubtedly lead to a higher degree of precision. For the purpose of computing averages, one more figure is retained in the results than could ordinarily be justified.

Table I. Analysis of Compounds of Known Halogen Content

Compound Analyzed	No. of Detns.		% Halogen Theory	% Halogen Found	Deviation Av.	Deviation Max.	Check Analysis[a]
Methyl chloride[b]	11	Cl	70.23	70.25	0.09	0.17	70.20
Dichloromonofluoromethane[c]	13	Cl	68.90	68.87	0.07	0.13	68.89
p-Bromotoluene[d]	14	Br	46.72	46.78	0.04	0.08	46.72
Chloral hydrate[e]	12	Cl	64.32	64.32	0.08	0.13	64.26
2-Chloro-2,3,3-trimethylbutane[f]	16	Cl	26.33	26.11	0.04	0.11	26.09
p-Bromoiodobenzene[g]	6	Br	28.25	28.19	0.04	0.08	28.20
		I	44.86	44.76	0.07	0.12	44.76

[a] Check analyses by peroxide bomb method.
[b] Commercial product, redistilled; boiled at −23.9° C., 744 mm.
[c] Commercial product, redistilled; boiled at 8.7° C., 738 mm.
[d] Purified by fractional crystallization; m. p., 26.5° C. to 27.5° C.
[e] Sublimed; m. p., 52° C. to 53° C.
[f] Recrystallized from petroleum ether; m. p., 130° C. to 131° C.
[g] Analyzed as received; m. p., 87° C. to 89° C.

One compound in Table I requires further comment. The *p*-bromoiodobenzene should perhaps not have been included, since it was not so pure as the listed results would indicate. The values for bromine and iodine were calculated by assuming equivalent quantities of the two halogens and simply dividing the titration value by two. Actually, however, the combined silver halides were recovered and weighed in each case, and the true value of each halogen was calculated from the titration value and the weight of precipitate. This method of determining two halogens together is not very good, since small experimental errors are magnified in the final values, but it showed 31.4 per cent bromine and 39.6 per cent iodine, which is approximately the halogen content of a mixture containing 89.6 per cent bromoiodobenzene and 10.4 per cent dibromobenzene. The compound was left in the table, however, because the peroxide bomb method gave almost identical results, and because it shows that the method is satisfactory for recovering total halogen.

Table II. Analysis of Various Organic Halogen Compounds

Compound Analyzed	No. of Detns.		% Halogen Theory	% Halogen Found	Average Deviation
Dichlorodifluoromethane, b. −30°	2	Cl	58.64	58.4	0.1
Ethyl chloride, b. 12°	2	Cl	54.95	53.0	0.0
Ethyl bromide, b. 38°	4	Br	73.33	73.1	0.1
Methyl iodide, b. 42.5°	3	I	89.41	89.4	0.2
Chloroform, b. 61°	3	Cl	89.08	88.2	0.2
Carbon tetrachloride, b. 76–7°	2	Cl	92.19	91.9	0.2
n-Butyl chloride, b. 78°	7	Cl	38.30	36.9	0.0
n-Amyl chloride, b. 108°	7	Cl	33.26	33.0	0.1
Chloropicrin, b. 112°	2	Cl	64.70	63.7	0.0
Tetrachloroethylene, b. 121°	3	Cl	85.51	85.2	0.2
n-Amyl bromide, b. 128–9°	4	Br	52.89	51.9	0.2
Ethylene chlorohydrin, b. 129°	2	Cl	44.03	43.2	0.0
Chlorobenzene, b. 132°	2	Cl	31.50	31.4	0.0
o-Chlorofluorobenzene, b. 235–6°	2	Cl	27.16	27.1	0.0
β-Chloroethyl chloroformate, b. 61–3° at 21 mm.	2	Cl	49.60	49.6	0.1
β-Chloroethyl acetate, b. 145°	3	Cl	28.93	30.0	0.0
Trimethylene bromide, b. 167°	3	Br	79.16	77.5	0.0
β,β′-Dichlorodiethyl ether, b. 178°	2	Cl	49.58	49.6	0.0
Benzyl chloride, b. 179°	2	Cl	28.01	27.7	0.1
o-Bromophenol, b. 194–5°	3	Br	46.18	45.8	0.1
Chloral alcoholate, m. 56–7°	3	Cl	54.98	54.7	0.0
Chloroacetophenone, m. 59°	2	Cl	22.93	23.1	0.0
Chloroacetic acid, m. 62–4°	2	Cl	37.52	37.3	0.0
p-Bromophenol, m. 63.5°	2	Br	46.18	46.3	0.0
p-Dibromobenzene, m. 87–8°	2	Br	67.75	67.8	0.0
Iodoform, m. 119°, sublimes	2	I	96.69	97.0	0.1
Hexabromoethane, m. 148–9°, sublimes	2	Br	95.24	95.2	0.0
Hexachlorobenzene, m. 227°, sublimes	3	Cl	74.70	73.9	0.0
Benzidine hydrochloride	2	Cl	27.57	27.5	0.0
Phenylhydrazine hydrochloride, m. 240–1°	2	Cl	24.52	24.4	0.1

The proposed procedure was also applied to a wide variety of other organic halogen compounds (Table II). Most of these substances were taken from stock reagent bottles and come were obviously not pure. The agreement with theory is close enough, however, to indicate that these compounds can all be analyzed successfully by this method. The method should be applicable to any substance which can be vaporized without too much pyrolysis. Failure to obtain satisfactory analyses was experienced in the case of two solid compounds (3,5-dinitrobenzoyl chloride and 2,6-dibromoquinonechloroimide), both of which decomposed on melting and left a carbonaceous residue which apparently retained some of the halogen.

Hydrogen or some other combustible gas may be used to sweep out the vaporized sample, but the city gas supply is considerably more convenient to use if it shows a negligible blank.

Attempts were made to determine fluorine in several organic compounds, using the same apparatus as for the other halogens. The results were always low and were somewhat erratic. This was not surprising, however, because the glass of the collecting chimney and of the inlet tube of the absorption flask was severely etched. But neither the vessel where the sample was vaporized nor the absorption flask itself was etched, and in the light of Cadenbach's success with apparatus made of silver (*1*), there is no apparent reason why the fluoro- compounds could not be analyzed satisfactorily by the proposed method if the collecting chimney and the inlet tube of the absorber were made of a suitable material, such as copper, silver, or lead. This possibility is under investigation at the present time. Especially encouraging is the fact that the two chlorofluoromethanes

and the *o*-chlorofluorobenzene listed in the tables yielded substantially correct values for the chlorine. This indicates that the decomposition of the samples containing fluorine is accomplished satisfactorily, and that the only remaining problem is that of recovering the fluorine quantitatively.

The time required for the analysis of a sample varies considerably, depending upon the physical characteristics of the substance. Excluding the time required for preparation of apparatus and standard solutions, but including the sampling and calculation of results, the analysis of a very volatile substance such as methyl chloride may be completed in 35 to 40 minutes, and may be turned out in a routine manner at the rate of one analysis every 15 minutes. Other substances may require as much as 30 to 45 minutes for the vaporization and burning of the sample. Much also depends upon the experience of the analyst.

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Determination of Sulfur Residues from Sulfur Application on Citrus Foliage

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Elemental sulfur (98 per cent sulfur by weight) mixed with a sulfur-free spray oil was applied to lemon leaves and then stripped off with purified carbon disulfide. After removal of the solvent, the sulfur was oxidized to inorganic sulfate by alkali fusion, and the sulfate ion was determined gravimetrically by precipitation as the barium salt. No special apparatus was required, and 10 to 200 micrograms of sulfur per square centimeter of leaf surface were determined rapidly and accurately in field practice.

ELEMENTAL sulfur has long been used for the suppression of various insect and mite pests and diseases of agricultural crops. It has been applied to the plants by different methods and in various combinations with inert materials and with other insecticides. The practice of applying elemental sulfur and petroleum spray oils simultaneously has brought up the difficulty of rapidly determining this elemental sulfur quantitatively in the presence of low concentrations of an oil of low volatility.

The usual procedure (*4*, *11*) has been to oxidize the elemental sulfur to sulfate, which is then precipitated with a soluble barium salt and weighed as barium sulfate. To simplify the procedure in work with spray residues, a preliminary extraction (*3*, *4*, *8*, *11*) of the sulfur from the plant material, followed by an oxidation process, is the most logical method. If the whole sample is oxidized in an alkaline fusion (*2*, *12*), the time element involved becomes prohibitive. This same objection applies, generally, to methods in which the elemental sulfur is reduced to sulfide (*6*).

Even when simplified as to size and heterogeneity of sample, the gravimetric methods are usually time-consuming. Several volumetric (*7*, *9*, *10*, *13*, *14*) and photometric (*15*) procedures for the estimation of the sulfate ion have been proposed, but these methods often require special equipment or are otherwise unsuited to the rapid determination of a small amount of elemental sulfur, in the presence of petroleum spray oils and spreading (wetting) agents, which are contained in a large amount of bulky material.

The present method is an outgrowth of a study initiated by Boyce *et al.* (*1*), who devised a sulfur-residue method involving a mechanical stripping of the sulfur from treated leaves in the absence of spray oils, and it combines the solvent extraction or stripping technique of Thatcher and Streeter (*11*) with an alkaline fusion similar to that of White (*12*). Wet-oxidation methods using nitric acid and bromine were found to give variable and incomplete oxidation (65 to 85 per cent) of elemental sulfur in the presence of a petroleum oil.

Experimental Procedure

The leaf sample to be analyzed (usually 125 average-sized mature leaves) was collected in a 2-quart Mason jar, which was capped and stored at 0° C. Just before the analysis, the leaves were removed from the jar with specimen forceps, and their surface areas were measured photoelectrically (*5*).

For the extraction of the sulfur, 100 ml. of recently redistilled carbon disulfide were added to the jar, and the sample was shaken vigorously for 2 minutes. After decantation of the supernatant liquid through a Büchner funnel, another 100-ml portion of carbon disulfide was added; the sample was shaken for 1 minute, and the supernatant liquid was decanted through the same funnel into the same receiving flask. The filtrate was transferred to a 250-ml. volumetric flask, and the funnel and receiving flask were washed carefully with 50 ml. of fresh carbon disulfide. This wash solution was then added to the previous filtrate, and the volume was adjusted to 250 ml. with additional carbon disulfide.

In the meantime, a fusion melt consisting of 8 parts of pure potassium hydroxide, 1 part of potassium nitrate, and 0.3 part of distilled water (all parts by weight) was prepared. This melt was crushed while warm and stored in a bottle tightly stoppered with a rubber stopper. For each sample, 3 grams of this crushed melt were weighed into a 20-ml. silver crucible, and an accurately measured 5-ml. aliquot of the carbon disulfide extract was pipetted onto the melt in the crucible. The carbon disulfide was evaporated from the sample, under vacuum, by placing the crucible containing the sample on a thin iron plate; a bell jar connected to the laboratory vacuum line was placed over the sample and the iron plate was warmed gently by means of a light bulb

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very sensitive hot plate. (If the rate of evaporation was too gh, spattering was likely to occur.) Under these conditions of aporation, the carbon disulfide apparently did not react with e fusion melt.

When all the solvent had been removed, the sample was fused er a shielded microburner with constant stirring with either a lver or a platinum wire. Considerable care had to be exersed in increasing the temperature of the melt during fusion, nce spattering was sure to occur if the temperature was raised o rapidly.

After bubbling had ceased, even over a raised flame, the ucible was heated strongly until the black deposit disappeared d the melt became water-clear. After cooling, the melt was ansferred to a 250-ml. beaker and dissolved in 10 ml. of disled water, and the excess potassium hydroxide in it was neutraled with 6 *N* hydrochloric acid, phenolphthalein being used as dicator. The resulting solution was filtered quantitatively to a 400-ml. beaker, and the filter paper was washed with four)-ml. portions of distilled water. The combined filtrate and ashings were brought to a volume of 300 ml. with distilled ater, and 1 ml. of 6 *N* hydrochloric acid was added.

After the acidified solution had been heated to boiling, 0.1 *N* rium chloride solution was added dropwise from a buret, with nstant stirring, until no new precipitate of barium sulfate rmed when the mixture was allowed to settle for a minute and drop of the barium chloride solution was added to a clear region the sample solution. The solution was kept just at the boilg point during this addition. An excess of 10 ml. of the barium loride solution was then added, and the sample solution was pt hot for 1 hour to allow time for complete precipitation of e barium sulfate. A watch glass was used to cover the beaker all times.

A Gooch crucible was prepared in the usual manner and dried 160° to 180° C. to constant weight. Immediately before tering, the sample was again tested for complete precipitation. precipitation was complete, the sample was filtered through e Gooch crucible, care being taken that no precipitate should here to the beaker or stirring rod. The precipitate was washed least ten times with hot water, until the washings were free m chloride ion.

The crucible and its contents were dried approximately 1 hour 160° to 180° C. to constant weight, and the sample was ighed as barium sulfate. Blanks were run on both untreated aves and solvent, and any necessary corrections were applied.

Discussion

The method reported in this paper is a combination of veral of the standard procedures for the determination of lfate ion, modified to eliminate certain errors introduced the extraneous materials commonly found in oil-spray eparations, and to expedite the oxidation of sulfur to lfate.

About 30 minutes, exclusive of drying periods, are required r one complete analysis; this value includes the time reired to measure the total surface area of the sample. By nning series of samples simultaneously and progressively, e authors have had no difficulty in analyzing 15 samples er man per 8-hour day.

In all test analyses, a known weight of yellow dusting sulfur 8 per cent sulfur by weight), comparable to that found in tual practice in the field on a unit leaf surface, was added a volume of a so-called "light-medium" sulfur-free spray l comparable to that applied in actual field work. This l-sulfur mixture was then applied to average-sized mature mon leaves, the leaf surface areas were measured photoectrically, and the sample was analyzed for micrograms of lfur per square centimeter of leaf surface. A small corction factor from blanks run on the solvent (1.1 mg. of lfur per 5 ml. of freshly distilled solvent) was applied; anks run on untreated leaves indicated that a small corction factor (average value of 1.5×10^{-2} mg. of sulfur er sq. cm. of leaf as used in these test analyses) was usually ecessary per unit leaf area, the magnitude of this correction ctor depending upon the previous spray history of the mpled leaves. Blanks run on the oil indicated it to be lfur-free. Results of analyses to show the accuracy and producibility of the method are presented in Table I.

Table I. Analyses of Elemental Sulfur on Leaf Samples

(125 leaves each, containing known quantities of pure sulfur and a sulfur-free spray oil[a], to show the accuracy and reproducibility of the method)

Sample	Sulfur Applied	Sulfur Recovered (Corrected)	
	Grams	*Grams*	%[b]
1	0.980	0.974	99.4
2	0.980	0.985	100.5
3	0.980	0.945	96.4
4	0.980	0.944	96.3
5	1.558	1.558	100.0
6	0.941	0.922	98.0
7	0.613	0.606	98.8

[a] Each sample contained 0.5 ml. of a so-called "light-medium" spray oil which had previously been shown to contain no sulfur, as determined by the present method.

[b] Coefficient of variation (per cent), 1.69.

It has been the experience of workers at the Citrus Experiment Station that a coefficient of variation of 1.69 per cent, as found in this method, indicates a low variability in methods to be applied to the removal of spray residues in field experiments.

This method has been extensively used at the University of California Citrus Experiment Station for the determination of sulfur residues resulting from field applications of various forms of sulfur in combination with other materials in controlstudies of the citrus thrips, *Scirtothrips citri* (Moult.), and the citrus bud mite, *Eriophyes sheldoni* Ewing. Greater precision, with a consequently increased time requirement, obviously may be attained in this method by increasing the number of extractions and thus the volume of extracting solvent. However, the degree of accuracy of the method as reported is thoroughly suitable for the purpose for which the method was devised.

Interfering Materials

The sulfur-free spray oil added to each sample (usually 0.5 ml. per 125 leaves) apparently did not interfere with the accuracy of this method. Excessive amounts of salts formed during neutralization, leading to coprecipitation, probably constitute the main source of positive error, while those forms of sulfur insoluble in carbon disulfide probably constitute the main source of negative error. Careful technique is essential in obtaining reproducible results with this method.

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Reductions with Nickel-Aluminum Alloy and Aqueous Alkali

Quantitative Determination of Halogens in Organic Compounds

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A RECENT publication (*3*) from these laboratories described the dehalogenation of organic compounds by a nickel-aluminum alloy and aqueous alkali.

Reduction as a method for the dehalogenation of organic compounds has been known for many years. The application of reduction methods for the quantitative determination of halogen in organic compounds is based on the conversion of halogen into salts of the halogen acids and the subsequent estimation of the halogen by standard analytical procedures. For this conversion, molecular hydrogen developed, for example, from zinc and acid is not generally applicable, since only a limited number of organic halogen compounds quantitatively undergo the halogen displacement when treated in this manner.

The introduction of catalytically activated hydrogen as an analytical tool for the dehalogenation of organic compounds was first described by Busch and Stove (*1*), who used a palladinized calcium carbonate catalyst which was prepared by heating an aqueous suspension of freshly prepared calcium carbonate with palladium chloride. The organic compound was dissolved in alcohol or water and then shaken at room temperature with the catalyst and hydrogen and sufficient alkali to neutralize the halogen acid formed. Later, Kelber (*2*) reported a similar procedure using a reduced nickel catalyst. Recently, the simultaneous reduction and dehalogenation of chloro-substituted azo compounds were reported to occur in the presence of Raney's nickel catalyst and hydrogen at room temperature and pressures varying from 1 to 3 atmospheres (*4*). All these methods when adapted for analysis require the use of hydrogen gas, a reduction catalyst prepared especially for the reaction, and the usual apparatus for catalytic reduction.

It was observed that on treatment with aqueous alkali and a nickel aluminum alloy, quantitative dehalogenation of organic compounds takes place (*3*). This reduction method has been very advantageously used for the quantitative determination of halogen in many aliphatic, aromatic, alicyclic, and heterocyclic compounds. The simple procedure and the fact that only the usual laboratory apparatus is required in carrying out the analysis give it a definite advantage over the other reduction methods for the determination of halogen in organic compounds. In addition, the results obtainable with this reduction method (100 ± 0.3 per cent) compare favorably in both accuracy and precision with those by any of the other reduction methods. Its advantages over the Carius method of heating with nitric acid in a sealed tube or the peroxide oxidation in a Parr bomb are obvious.

Procedure for Analysis

For Alkali-Soluble Compounds. A 0.3-gram sample of the substance is dissolved in 100 ml. of 5 per cent sodium hydroxide in a tall 500-ml. beaker. Using a watch glass to cover the beaker, 3 grams of Raney nickel-aluminum alloy, in three or four portions, are added in the course of 10 minutes. The beaker must be covered with the watch glass at all times during the reduction to prevent loss of the solution by the fine spray caused by the liberation of hydrogen. The alloy may be added by simply sliding the watch glass just over the lip of the beaker, dropping the charge into the solution, and restoring the watch glass to its original position. When the reaction has subsided, the solution is heated on a steam bath for 15 minutes, the condensate on the watch glass is quickly washed into the beaker, and the solution is heated for about one hour at 90° C. to 95° C.. At this stage, the nickel settles out and a clear colorless solution is obtained.

The condensate on the watch glass is again washed into the beaker and the alkaline solution is decanted from the nickel into a 200-ml. volumetric flask. The residual nickel is washed three times by decantation with hot water, the solution and washings are combined, and allowed to cool, and the volume is adjusted to the mark. A 100-ml. aliquot, or a fraction thereof, is acidified to Congo red paper with 10 per cent nitric acid and the halogen determined by any standard procedure. If the halogen is to be determined gravimetrically, the acidified solution is cooled and filtered if necessary, and the halide is then precipitated.

For Alkali-Insoluble Compounds. The sample is weighed into a 250-ml. Erlenmeyer flask equipped with a condenser. After adding 10 ml. of 95 per cent ethyl alcohol and 3.5 grams of alloy, the condenser is set in place and the flask cooled in an ice bath. Seventy-five milliliters of cooled 10 per cent sodium hydroxide are added through the condenser and, after the initial reaction has subsided, the ice bath is removed and the flask heated with a low flame until all the alloy has reacted (1.5 to 2 hours). It is not advisable to heat the mixture too rapidly since the initial reaetion is vigorous and solution may be lost through the condenser. Any excessive foaming, during the reflux period, is controlled by the addition of a few drops of octyl alcohol. When the reduction is completed, the condenser is washed down with water and the determination completed as described for the alkali-soluble compounds.

Table I. Determination of Halogen

Halogen Compound	Theory %	Found %
3,5-Diiodo-4-hydroxyphenylacetic acid[a]	62.84	62.15
β-(3,5-Diiodo-4-hydroxyphenyl)-α-phenylpropionic acid[a]	51.40	51.61
α-(3,5-Diiodo-4-hydroxyphenyl)-β-phenylpropionic acid[a]	51.40	51.60 51.50
N-methyl-3,5-diiodochelidamic acid[a]	56.57	56.54 56.44
β-Bromopropionic acid	52.21	52.38 52.06
Dibromolevulinic acid[a]	58.32	58.00
Bromobenzene	50.85	51.01 50.87
2-Bromopyridine	50.61	50.76
p-Bromoacetophenone	40.17	40.17 40.40
2-Bromocholestanone[a]	17.17	16.34 16.59
Cholesterol acetate dibromide[a]	27.35	27.56 27.36
m-Chlorobenzoic acid	22.67	23.04 23.06
p-Nitrochlorobenzene	22.52	22.57 22.68
β-(*p*-Chlorobenzoyl)-propionic acid[a]	16.73	17.01 16.81
2-Chloropyridine	31.25	31.02

[a] Compounds prepared in the authors' laboratory; others were Eastman Kodak chemicals purified by distillation and/or recrystallization.

Summary

A simplified procedure for the determination of halogen in organic compounds is described. Quantitative displacement of halogen by catalytically activated hydrogen occurs when the organic compound is treated with a nickel-aluminum alloy in an alkali hydroxide solution. The halogen is obtained in the form of the alkali salt of the halogen acid and can easily be estimated

by any standard procedure. The method is accurate, rapid, and adaptable to routine analysis, requiring only the simplest of laboratory glassware and no special reagents.

Acknowledgment

The authors wish to take this opportunity to thank Felix J. Pheiffer of the Analytical Division for his help with the preliminary work.

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Analysis of Ethanol for Minor Amounts of Esters and Aldehydes

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ALTHOUGH often of a routine nature, the estimation of esters and aldehydes in 95 per cent alcohol is fraught with difficulties. Furfural, acidity, and permanganate-time tests are rather readily made while specific gravity determination requires only careful attention to detail. Higher alcohols present some difficulties not considered here.

Esters

The ester content of pure ethanol usually lies between 1 and 5 parts per 100,000 volumes calculated as ethyl acetate on a 100-proof basis. By the A. O. A. C. method for esters in distilled liquors (*1*) on a 50-ml. sample, this would be equivalent to 0.05 to 0.3 ml. of 0.1 *N* alkali. In distilled liquors except gin the amount is usually about 10 times that cited.

The usual chemical glassware is not sufficiently alkali-resistant to be used in the determination of such small quantities of esters. The alkali used to saponify the esters attacks the glass to such an extent that the alkali so consumed far exceeds that necessary to saponify the esters. Consequently the blank determinations are too high for accurate work. Furthermore, such blanks vary with the age of the glassware used.

A few pieces of old glassware were found in this laboratory which gave small blanks, probably owing solely to usage; they are used in pairs, giving similar blanks. An old expedient of using silver flasks involves washing out the contents into a clean flask after saponification and before titrating, using freshly boiled and cooled distilled water.

Recent studies of the chemical resistance of borosilicate glasses sold as Pyrex, Kimble, and Glasbake (*3*) show that dilute potassium hydroxide solutions attack such glass to a lesser degree than dilute sodium hydroxide solutions. Therefore, potassium hydroxide is used. Special alkali-resistant Pyrex, while not fully satisfactory, is preferable to the usual chemical glass.

Procedure. Measure 50 ml. of 95 per cent ethanol and dilute to 100 ml. with freshly boiled and cooled distilled water. Exactly neutralize any free acidity with 0.02 *N* potassium hydroxide solution, using phenolphthalein as indicator. Add exactly 10.0 ml. of 0.1 *N* potassium hydroxide solution and reflux gently for 30 minutes. Wash down the condenser with neutral distilled water, stopper and cool the flask and contents in a water bath, remove the stopper carefully, and wash down. Add exactly 10.0 ml. of 0.1 *N* hydrochloric acid solution and titrate the excess acid with 0.02 *N* potassium hydroxide solution. Run a blank determination under identical conditions, substituting 100 ml. of freshly boiled and cooled distilled water for the diluted alcohol. Each milliliter of titration is equivalent to 0.00176 gram of ethyl acetate. The usual titration will therefore fall between 0.6 and 2.9 ml. of direct titration after subtraction of the blank.

Aldehydes

The volumetric A. O. A. C. method, involving titration of excess bisulfite with standard sodium thiosulfate, is simple and sufficiently accurate when used for one or two samples. As a special distillation is required for each sample for multiple determinations, a colorimetric method is preferred for economy of time.

In the most commonly used colorimetric method (*2*) aldehyde-free alcohol is prepared by distillation from metaphenylenediamine hydrochloride. Unless freshly prepared with great care, error is easily introduced, and the alcohol is rarely completely aldehyde-free. This may be due in part to contact with air in redistillation and in storage, even in a cold, dark place. Redistilled synthetic methanol is consistently aldehyde-free and has been used largely to replace the ethyl alcohol in this determination for several years. While distilled liquors except gin are apt to contain 4 to 12 parts per 100,000 of aldehyde, the amounts in alcohol are of the order of 0.05 to 0.2 part per 100,000.

Procedure. To 2.5 ml. of the alcohol sample add 2.5 ml. of water and 15 ml. of 50 per cent by volume redistilled synthetic methanol. Add 5 ml. of fresh Schiff's reagent prepared according to A. O. A. C. standards, and mix at once. All solutions must be at 60° F. and, after mixing, maintained at that temperature for 20 minutes to develop the maximum color due to the presence of aldehyde.

Prepare standards as follows: To 5 ml. of 50 per cent aldehyde-free alcohol add 15 ml. of 50 per cent synthetic methanol plus 0.02, 0.025, 0.03, 0.05, and 0.075 ml. of freshly diluted acetaldehyde solution containing 0.0001 gram per ml. Such small quantities can be measured with a sufficient degree of accuracy by using a 1-ml. pipet graduated in 100 parts. Vary these standards to suit specific needs. To each of the above add 5 ml. of the Schiff's reagent, mix at once, and treat as with the sample. Pyrex test tubes (25-ml.), thoroughly cleaned with chromic acid–sulfuric acid solution and efficiently rinsed, are convenient comparison tubes.

Compare the samples and the standards, all made at approximately the same time, after standing for 20 minutes at 60° F.

Summary

In the accurate determination of 1 to 5 parts per 100,000 of esters in 100-proof ethanol it is necessary to have either well-aged glassware which gives a small blank, or silver flasks. Many difficulties in the determination of 0.1 part per 100,000 of aldehyde are eliminated by substituting redistilled methanol for the major part of the aldehyde-free ethanol.

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Removal of Air from Powders in Density Determination

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IN THE determination of the density or specific gravity of fine powders by the method of immersion in a liquid in a pycnometer, it is often difficult to remove the air from the spaces between particles, even though the sample be of a material that in coarser form is easily wetted by the immersion liquid. The air is commonly removed by keeping the open pycnometer, containing the sample and enough liquid to cover it well, in an evacuated space while the air comes off in rarefied bubbles. This process seldom goes through smoothly; the bubbling is spasmodic, sometimes coming in unanticipated bursts that throw part of the sample out of the bottle. The last bubbles of air hang on tenaciously, and considerable agitation is required to shake them out from the interior of the powder bed. This part of the work is commonly handled by alternate stirring and evacuation, which often proves to be a tedious operation.

Previous workers have introduced between the pycnometer and the pump a tube designed to catch any particles unavoidably thrown out from the pycnometer; the material thus caught was weighed and allowance made for it in the calculation (*1*). The advantages of using a suitable wetting liquid have been cited by Nutting (*2*). Where water is used to immerse organic crystalline compounds, it is often necessary to add a wetting agent.

With the setup described below the air can be removed smoothly by controlled agitation while the sample is under vacuum. The method has been used in this laboratory for several months.

The apparatus, shown in Figure 1, is simple and may be assembled from readily available elements. The source of agitation is a vibrator, *V*, of the type sold for massaging the face and scalp. In place of the original vibrator head is a special head, *H*, which holds the pycnometer flask, *F;* this head consists of a one-hole rubber stopper in which three nails are inserted at a slight angle to grasp the body of the flask. The vibrator is mounted head upward on a heavy base, *B*, which rests on a thick rubber sheet, *G*, covering the top of a wooden block, *W*. The rubber sheet serves as a gasket between the block and a vacuum bell jar, *J*, which covers that part of the assembly so far described. One or more air holes, *A*, are cut in the gasket to prevent the central portion from being lifted when the jar is evacuated. A round-bodied electric lamp cord (Type SJ) is run from the vibrator down through the gasket into the block and out at one side, the passage through the wood being bushed airtight with a rubber stopper, *S*. For convenience in handling there should be a coupling, *C*, in the line between the vibrator and the gasket.

Adjustment of the vibration intensity and of the pressure reduction is provided by the following arrangement: The apparatus is plugged into a continuously variable transformer, *T*, with voltage range from 0 to 115 or slightly above. The rubber tube from the bell jar to the house vacuum line has a branch connected to a manometer, *M*, and another branch left open at the end, *E*. With one hand on the adjusting knob of the transformer and the index finger of the other hand resting lightly over tube end *E*, the operator has fingertip control over vibration and vacuum at all times. If it is desirable to maintain a particular adjustment for some time without attention, the transformer may be set for the necessary voltage and the vacuum kept fairly constant by leakage through a screw pinchcock, *P;* or if the maximum vacuum is desired, the end of a cork stopper may be left resting against *E*.

A little practice will enable one to judge readily the optimum adjustments of agitation and vacuum, both of which may need considerable variation during the treatment of a given sample. The ideal is to maintain continuous but not violent bubbling, until not enough air is left to affect sensibly the measurement of the sample volume. A test for completeness of air removal in this sense is to observe whether the height of the liquid in the pycnometer changes on application or removal of vacuum. The test is most sensitive when the full vacuum is suddenly released, as by jerking the finger away from *E*, and when the bottom of the meniscus is nearly in line of sight with some letter or other marking on the flask.

When the last significant traces of air are persistent, two means are available for hastening their removal. One is to stir the sample with a stiff wire rod, *R*, extending through a flexible rubber stopper in the top of the bell jar. Another way, which is more convenient and may prove adequate, is to jerk the flask repeatedly by jiggling the electric plug that connects the transformer to the house line.

The current should not be left on longer than necessary, lest in its vacuum-jacketed condition the vibrator should become overheated. The time required for the whole operation of air removal is usually only a few minutes.

The density values obtained by this method are equivalent to those by the more common process, for in both cases the air removal is carried to the same end point as indicated by the expansion-contraction test. The saving of time by the method here described may be negligible or large, depending on the difficulty of manipulation of the given sample by the older method. The principal advantages, evident particularly with the more troublesome samples, are convenience and relative safety from loss of sample by spurting. The term

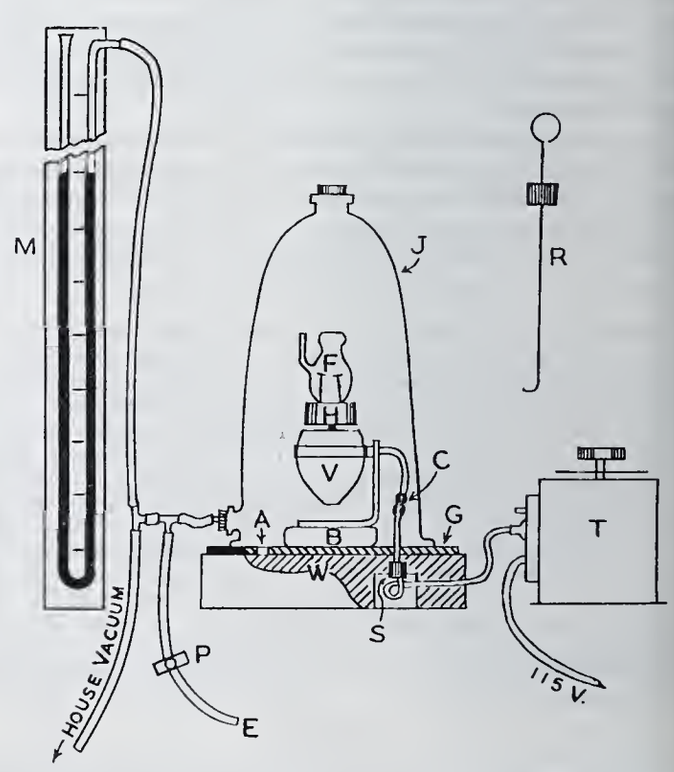

FIGURE 1

"relative safety" is used advisedly, for the method is not foolproof. The chief function of the vibration is to loosen the bubbles while they are expanding, thereby permitting them to come off in a comparatively steady stream rather than build up to a condition that has to be relieved by a concentrated outburst. The vibration, judiciously applied, is thus a means of safeguarding against accidents that otherwise are difficult to avoid.

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Determination of Sugars in Apple Tissue

Some Modifications of the Usual Procedures

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NUMEROUS methods are available for extracting and determining the sugars found in plant tissues. Many of these have been reviewed recently by Browne and Zerban (*5*) in their excellent treatise on sugar analysis.

The Association of Official Agricultural Chemists recommends 50 per cent alcohol for the extraction of sugars from grain and stock feeds (*2*) and 80 per cent alcohol for the removal of sugars from plants (*1*). Jackson and McDonald (*8*) used the volumetric dichromate–ferrous ammonium sulfate–phenanthroline method for cuprous oxide and later reported (*9*) a study of Munson and Walker's tables for reducing sugars. Erb and Zerban (*7*) combined the Munson and Walker procedure for reducing sugars with the Jackson and Mathews (*10*) method for levulose, in a procedure for the individual determination of dextrose and levulose in a mixture containing sucrose. Erb and Zerban used a table of modified Munson and Walker values and calculated the variable reducing factors for different ratios of dextrose and levulose. Stegeman and Englis (*12*) compared several volumetric procedures for determining cuprous oxide with the gravimetric method and discussed briefly the possible use of diphenylamine with dichromate in sugar work.

The rupture of cell structure before extracting is advantageous. Kneen and Blish (*11*) heated wheat plants for 15 minutes in an oven held at 140° C. before extracting the sugars. The use of the Waring Blendor for sample preparation has already been described (*6*) and its application is rapidly being extended.

Experimental

RECOVERY OF REDUCED COPPER. In an investigation of the sugars in apple tissue preliminary trials with several methods led the authors to consider the possibility of using diphenylamine with dichromate, for the recovery of reduced copper, as suggested by Taran (*13*). Table I shows the values obtained for the recovery of copper from varying weights of cuprous oxide by this procedure, the details of which are described later.

TABLE I. RECOVERY OF REDUCED COPPER
(From cuprous oxide dissolved in ferric sulfate solution by titration with potassium dichromate and diphenylamine indicator)

Copper Added	Copper Recovered	Recovery
Mg.	*Mg.*	%
42.4	42.6	100.4
64.5	64.8	100.5
67.7	67.7	100.0
82.2	83.1	101.1
87.2	87.7	100.5
89.7	89.4	99.7
91.4	91.9	100.6
98.8	98.6	99.8
100.1	99.5	99.4
107.9	107.1	99.3
109.8	110.8	100.9
121.9	121.9	100.0
130.7	133.1	101.9
135.5	136.1	100.4
		Mean 100.32 ± 0.18

In another series of trials varying quantities of cuprous oxide obtained by the Munson-Walker procedure were weighed, dissolved, and titrated directly by the dichromate–ferrous ammonium sulfate–*o*-phenanthroline–ferrous sulfate method of Jackson and McDonald and by the dichromate–ferric sulfate–diphenylamine procedure herein described. Table II gives the results of these trials.

TABLE II. COMPARISON OF JACKSON AND MCDONALD AND DICHROMATE–FERRIC SULFATE–DIPHENYLAMINE METHODS FOR RECOVERY OF REDUCED COPPER

Method of Jackson and McDonald			Dichromate–Ferric Sulfate–Diphenylamine Method		
Copper Added	Copper Recovered	Recovery	Copper Added	Copper Recovered	Recovery
Mg.	*Mg.*	%	*Mg.*	*Mg.*	%
29.7	30.2	101.7	..	..	..
50.0	49.5	99.0	..	..	..
55.1	55.0	99.8	39.7	39.5	99.5
74.8	75.3	100.7	45.4	45.8	100.9
91.7	91.3	99.6	49.2	48.6	98.8
96.0	96.1	100.1	91.9	91.2	99.2
135.3	134.8	99.6	128.7	127.8	99.3
179.3	178.8	99.7	136.8	136.8	100.0
182.3	181.0	99.3	140.0	140.7	100.5
232.0	230.8	99.5	184.3	185.9	100.9
254.6	254.6	100.0	225.2	227.0	100.8
266.5	268.1	100.6	318.2	319.5	100.4
	Mean 99.97 ± 0.21			Mean 100.03 ± 0.25	

EXTRACTION. Several procedures were tried for convenience and thoroughness of extraction. Quadruplicate samples were analyzed raw and after being heated for 15 minutes in an ordinary convection oven set for 140° C. Although the differences were small, the heated samples averaged 7.06 per cent for reducing sugars and 1.66 for sucrose as compared with 6.71 and 1.63, respectively, for the unheated samples.

The slight gain in reducing sugars was attributed to a more thorough extraction of the heated sample. However, additional trials were run to ensure absence of pronounced changes in the distribution of the sugars present.

For this purpose triplicate samples of 70 to 80 grams each, of Rome Beauty tissue, in 10-cm. (4-inch) aluminum dishes with tight covers, were heated for periods of 5, 10, and 15 minutes in an ordinary convection oven set at 110°, 120°, and 140° C. A similar series was run with duplicate samples from a different stock sample of Rome Beauty tissue, but the heating was accomplished in a forced draft oven set for the same range of temperatures and 15-minute heating periods only. Unheated samples were analyzed at the same time for comparison. The average values for both series are shown in Table III.

The original procedure used to extract the apple tissue with alcohol consisted in two hand grindings in a glass mortar, but trials showed that the operation could be accomplished

Table III. Sugar Content of Heated and Unheated Tissue of Rome Beauty Apples

Heating Period	Temp.	Convection Oven: Reducing sugar (as invert)	Convection Oven: Sucrose (as invert)	Convection Oven: Total sugar	Forced Draft Oven: Reducing sugar (as invert)	Forced Draft Oven: Sucrose (as invert)	Forced Draft Oven: Total sugar
Min.	° *C.*	%	%	%	%	%	%
Not heated		7.58	1.53	9.11	7.88	1.88	9.76
5	110	7.54	1.71	9.25	....	....	....
10	...	7.73	1.55	9.28	....	....	....
15	...	7.61	1.71	9.32	7.82	1.97	9.79
5	120	7.68	1.61	9.29	....	....	....
10	...	7.63	1.48	9.10	....	....	....
15	...	7.72	1.32	9.04	7.86	1.86	9.73
5	140	7.67	1.52	9.19	....	....	....
10	...	7.53	1.43	8.96	....	....	....
15	...	7.49	1.44	8.93	7.93	1.79	9.72

The average water loss in the forced draft oven was 0.56, 0.88, and 4.43 per cent, respectively, at 110°, 120°, and 140°, for 15 minutes; in the convection oven the losses were much less, ranging from 0.02 to 0.48 per cent.

more quickly and with equal efficiency by the use of the Blendor. With triplicate samples of Grimes Golden tissue, two extractions in the Blendor gave an average of 5.56 per cent total reducing sugars as compared with an average of 5.23 per cent for one extraction in the Blendor and 5.29 per cent for two extractions in the glass mortar.

Clarification. The need for clarification of alcoholic extracts of plant tissues is an open question and probably depends in considerable degree upon the nature of the plant material from which the sugars are extracted.

In the present work triplicate or quadruplicate trials with alcoholic extracts of tissue of six varieties showed little or no appreciable difference in total reducing sugars between cleared and uncleared samples. The same was true for sucrose where this sugar was determined. On the other hand, one variety (Gravenstein) did show a higher value for reducing sugar in the uncleared sample (Table IV). In each instance the samples were cleared with neutral lead acetate and disodium phosphate and each lead precipitate was washed twice by centrifugation.

Table IV. Effect of Clearing Alcoholic Extracts from Seven Varieties of Apples

Variety	Month Analyzed	Reducing Sugar (as Invert Sugar): Not clarified	Reducing Sugar: Clarified	Sucrose (as Invert Sugar): Not clarified	Sucrose: Clarified
		%	%	%	%
Gravenstein	September	6.95	6.76	1.70	1.70
Grimes Golden	September	9.53	9.48	3.67	3.60
Delicious	November	9.91	9.87	....	....
Starking	December	9.34	9.32	....	....
Stayman Winesap	December	6.99	6.97	....	....
York Imperial	December	5.10	5.07	....	....
Rome Beauty	March	6.97	6.94	....	....
Rome Beauty	April	7.53	7.53	1.89	1.86

Several trials were made on the method of clearing alcoholic extracts or fresh juice when the cuprous oxide was determined by the dichromate-diphenylamine method (Table V). In all instances neutral lead acetate was used as the clearing agent. Comparisons were made of sodium oxalate, disodium phosphate and sodium carbonate as deleading agents, and of filtration and centrifugation of lead precipitates.

The clarification of alcoholic extracts of ground apple tissue proved to be slightly more difficult than that of apple juice and necessitated 2.5 ml. of lead acetate solution and 2.0 ml. of disodium phosphate solution. Trials were made to determine the effect of excess disodium phosphate and of alcohol in the clarified extracts. A 5-ml. excess of phosphate solution (total of 7 ml.) and 15 ml. of 85 per cent alcohol were without appreciable effect on the results obtained.

Information was desired concerning the sugar values obtained by combining the several steps tried individually, as compared with procedures already accepted as suitable for sugar work. For this purpose the combined procedure was compared with the A. O. A. C. (*1*, *2*) methods mentioned.

A composite stock sample was made from 24 Rome Beauty apples and run through a food chopper. The material was mixed well and three lots were weighed out rapidly, in duplicate, for analysis. The first lot was extracted and analyzed in accordance with A. O. A. C. directions for plants, the second lot was extracted and analyzed in accordance with A. O. A. C. directions for grain and stock feeds, and the cuprous oxide values from both lots were determined gravimetrically after clarification with neutral lead acetate by filtration. The third lot was heated in aluminum dishes, comminuted and extracted in the Blendor, and clarified by centrifugation, and the cuprous oxide was titrated with dichromate and diphenylamine. The average values for this comparison are given in Table VI.

Finally, a series of six replications was made to test the reproducibility of the values obtained by the modified procedure. Dextrose and levulose were determined individually by the method of Erb and Zerban (*7*) and sucrose was determined by the A. O. A. C. (*3*) method after inversion with hydrochloric acid at room temperature. The results of these replications are shown in Table VII.

Discussion

Table I shows that copper in the form of cuprous oxide can be determined satisfactorily by dissolving in ferric sulfate solution and titrating with potassium dichromate with diphenylamine as an indicator, and Table II indicates that the values so obtained compare favorably with those obtained by the volumetric method of Jackson and McDonald. In the former method the cuprous oxide dissolves readily, and the end point is distinct and with a little practice is easily recognized.

The heating of apple tissue prior to comminution and extraction may or may not be advantageous, depending somewhat upon the physical nature and degree of ripeness of the fruit. Hard, tough, or immature fruit is more easily prepared and extracted if first heated. This softens the tissue, ruptures the cells, and allows uniformity in sampling. It also destroys enzymes and, if carefully done, does not hydrolyze the sucrose present. Ripe fruit softened by maturity is not benefited by preliminary heating. Table III indicates that Rome Beauty apple tissue may be heated for periods of 5 to 15 minutes at temperatures of 110° to 140° C. without pronounced effect upon the values for reducing sugars and sucrose. In this instance the fruit was mature and the heating of little or no advantage. An interesting observation on the comparison of heated and unheated samples was the apparent greater amount of oxidation in the unheated samples as evidenced by their dark color in contrast to the lighter color of the heated samples. In storage at 3° C., the heated samples kept over a longer period than the unheated samples without appreciable change in sugar values.

The data relating to clarification are interesting (Table IV). Of the seven varieties tried, differences between clarified and unclarified extracts could perhaps be ignored in all cases except that of Gravenstein. Nevertheless, in no case did the value for reducing sugars in the clarified extract exceed that for the unclarified and the general trend is toward

Table V. Values for Reducing Sugars in Apples as Affected by Methods of Clarification

Deleading Agent	Method of Removing Ppt.	Made to Volume before or after Clarification	No. of Washes for Each Lead Ppt.	Mg. of Reducing Sugars as Invert Sugar in 5-Ml. Aliquot of Sample: Juice	Extract 1	Extract 2
at'd $Na_2C_2O_4$	Centrifugation	After	1	110.8	..	..
	Centrifugation	After	None	105.5	..	..
at'd Na_2HPO_4	Centrifugation	After	1	110.3	..	..
	Centrifugation	After	None	106.6	..	..
Dry Na_2CO_3	Filtration	Before	...	...	83.3	..
Dry Na_2HPO_4	Filtration	Before	...	...	82.5	..
at'd Na_2HPO_4	Centrifugation	After	3	...	84.5	..
	Centrifugation	After	None	...	74.4	..
Dry Na_2CO_3	Filtration	Before	...	...	..	75.0
Dry Na_2HPO_4	Filtration	Before	...	...	..	75.1
	Centrifugation	Before	...	...	..	75.6
Not clarified	...	...	...	...	..	75.5

lightly higher values in the nonclarified material. It is possible that stage of maturity and relative amount of chlorophyll-bearing tissue are more important than varietal difference in this respect. It may or may not be significant that most of these trials were made during the winter and spring months, on apples taken from storage, whereas the largest differences between clarified and nonclarified extracts occurred in the analyses made earlier in the season. More data are needed on this point.

As indicated in Table V, sodium oxalate, disodium phosphate, or sodium carbonate may be used as a deleading agent in the ferric sulfate–dichromate–diphenylamine method. Since 5-ml. excess disodium phosphate did not disturb the values for reducing sugars it was accepted as a deleading agent. If the sugar solutions are made to volume after clarification it is necessary to wash the lead precipitates in order to avoid low values for sugar. If desired, the lead precipitates may be removed by centrifugation rather than by filtration, in which case it is convenient to make the solutions to volume, centrifuge in a closed tube, and omit the washing process.

Uncleared solutions may be somewhat less satisfactory to pipet, as particles may tend to gather in the tip or cling to the inside walls of the pipet, but where clarification can be omitted there is a very material saving in time. The results of these trials indicate that for ordinary work the clearing process may be omitted with apple extracts in most instances.

Table VI shows over-all values obtained from the modified procedure in comparison with those obtained by the two variations of the A. O. A. C. methods, and Table VII shows the values from six replications when dextrose, levulose, and sucrose were determined individually. These results indicate that the modified procedure is suitable for the analysis of apple tissue.

The Waring Blendor has been found to be very effective in reducing and mixing either raw or heated apple tissue in this work. Extraction of the tissue with alcohol, also, may be accomplished quickly and easily by use of the Blendor, the time required for two extractions in the blendor being approximately one half that required for two extractions by hand grinding in a mortar. Other time records show that omitting the time required to evaporate the extracts on the steam bath, which is similar in both methods, the time necessary for the determination of total reducing sugars on quadruplicate samples was 3.3 hours for the modified procedure herein proposed as compared with 5 hours by the A. O. A. C. procedure for feeds. Extraction in the Blendor, clarification in the centrifuge, and titration of the copper oxide by the dichromate-diphenylamine method each requires approximately one half the time necessary for extraction on the steam bath, clarification by filtration, and gravimetric determination of the copper oxide, respectively. Extraction by Soxhlet apparatus is still more time-consuming than extraction on the steam bath.

Procedure

Preparation of Extracts. Cut the sample into slices, weigh, heat in a tared aluminum dish with tight cover for 15 minutes in an oven set for 140° C. to soften the tissue, cool, and transfer the material to the tared Blendor cup with the aid of water. (The heating may be omitted with soft tissue which reduces readily in the Blendor cup.) Weigh the mixture in the Blendor cup to determine the amount of water added beyond the weight of the original sample before it was heated. The weight of water used for the transfer is usually about one third that of the sample taken for heating and the small amount of water lost in heating is made up in the transfer to the Blendor cup. Comminute thoroughly and store as a bulk sample in the refrigerator. Heated apple tissue will remain unchanged for days or weeks and unheated tissue will keep for a somewhat shorter period.

Extract 20 to 30 grams in the Blendor with 1 or 2 grams of calcium carbonate and approximately 150 ml. of 95 per cent alcohol; this results in a mixture above 70 per cent alcohol and the separation of a flocculent precipitate. Filter the mass on a 9-cm. Büchner funnel into a 500-ml. suction flask, remove the paper and residue to the Blendor cup, and extract again with about 100 ml. of alcohol, again filter, combining the second filtrate with the first and washing the residue with alcohol until the total volume approximates 300 ml. Transfer the extract to a 400-ml. beaker with the aid of a little water, add a small amount of calcium carbonate, and evaporate on the steam bath to a volume of 5 to 10 ml.

If clarification is omitted, transfer the sirupy residue from the steam bath directly to a 100-ml. flask and make up to volume for analysis. If clarification is desired, add 2 ml. of saturated lead acetate solution to the flask and make to volume. Mix well, allow to stand a few minutes, and transfer all (or most) of the sample to a closed, dry centrifuge tube. Centrifuge until clear (5 to 10 minutes) and pour off into a second dry centrifuge tube containing an amount of dry disodium phosphate sufficient to precipitate all the lead present. Mix well, close the tube to prevent evaporation, and again centrifuge until clear.

Pour off and retain the clear liquid for analysis. A somewhat longer and possibly slightly more accurate procedure for clarification involves washing the lead precipitates twice or three times by centrifugation before making the sample to definite volume for analysis.

Determination of Sugars. Determine reducing sugars, dextrose, and levulose by the combined procedure of Erb and Zerban (7) and sucrose by the A. O. A. C. (4) method, after inversion with hydrochloric acid at room temperature. Filter the cuprous oxide on a loose-bottom (Caldwell) crucible, transfer the crucible to a 400-ml. beaker containing 50 ml. of ferric sulfate solution, dissolve the cuprous oxide, rinse, and remove the crucible and add 20 ml. of sulfuric acid. Add 3 or 4 drops of

Table VI. Comparison of the Modified Procedure with A. O. A. C. Methods for Sugars in Apple Tissue

Procedure	Reducing Sugars (as Invert) %	Sucrose (as Invert) %	Total Sugar %
A. O. A. C. method for plants	7.34	1.75	9.09
A. O. A. C. method for feeds	7.31	1.75	9.06
Modified procedure	7.53	1.86	9.39

Table VII. Sugar Content of Replicate Samples of Apple Tissue

Replication No.	Dextrose %	Levulose %	Reducing Sugars %	Sucrose (as Invert) %	Total Sugar %
1	1.03	4.60	5.63	0.77	6.40
2	1.13	4.51	5.64	0.68	6.32
3	1.13	4.49	5.62	0.56	6.18
4	1.15	4.51	5.66	0.55	6.21
5	1.16	4.55	5.71	0.60	6.31
6	1.25	4.47	5.72	0.62	6.34
Mean	1.14 ± 0.03	4.52 ± 0.02	5.66 ± 0.02	0.63 ± 0.03	6.29 ± 0.03

diphenylamine indicator and titrate with dichromate solution until a darkening occurs. At this point add 5 ml. of phosphoric acid and 8 to 10 drops more of indicator. Add water to make the total volume about 250 ml. and complete the titration to the first permanent violet coloration.

REAGENTS. Reagents (Fehling's and Ost's) for reducing sugars are prepared according to A. O. A. C. (*4*) directions.

Ferric sulfate solution is made by dissolving 60 grams of the hydrated salt [$Fe_2(SO_4)_3.9H_2O$] in 1 liter of solution and filtering. The solution must be free from ferrous ions.

Potassium dichromate is weighed to give exactly 7.7135 grams per liter of solution. One milliliter is equivalent to 10 mg. of copper.

Diphenylamine is used as 0.2 per cent solution in concentrated sulfuric acid.

Sulfuric acid is a 1 to 7 aqueous solution.

Orthophosphoric acid is the usual 85 per cent U. S. P. sirup.

Lead acetate solution is a saturated solution of the neutral salt.

Disodium phosphate is used either dry or as a saturated solution.

Summary

In the analysis of apple tissue for sugars it is advantageous to heat the tissues in closed aluminum containers before grinding, to disintegrate, mix, and extract the heated tissue in a Waring Blendor, and to determine cuprous oxide by dissolving it in ferric sulfate solution and titrating with potassium dichromate in the presence of diphenylamine indicator. The procedure is rapid and easily manipulated and the results are sufficiently accurate for the determination of sugars in many plant materials.

In most instances it appears safe to omit clarification of the alcoholic extracts of apple tissue, with a corresponding saving in time. Where clarification is necessary this can be accomplished satisfactorily by addition of neutral lead acetate and centrifugation as described.

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Simple Device for Preparing Vapor-Air Mixtures

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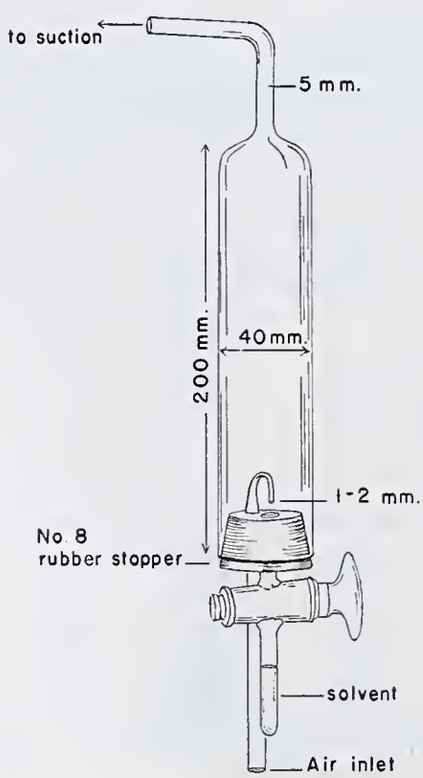
IN TESTING the efficiency of a combustion apparatus for chlorinated hydrocarbons in the atmosphere, the writer desired to introduce into the unit known amounts of solvent vapor at rates of 1 to 2 liters per minute. A simple method of doing so, and one most nearly meeting this need, was found in the experimental work of Olsen, Smyth, and co-workers (*1*). However, a modification which would ascertain by successive weighings the amount of solvent used in each run seemed desirable. A very satisfactory method was finally devised which gave precise weighings and a wide range of concentrations and required very little manipulation.



Both ends of a two-way stopcock of about 8-mm. bore were cut off about 35 mm. from the base and one end was sealed. A short piece of glass tubing, 7 mm. in inside diameter, was drawn out, bent, and inserted in the two-hole rubber stopper as shown. The mixing chamber was a filter tube, the neck of which was bent at a right angle and connected to the combustion unit. The apparatus was mounted by means of a clamp on a ringstand.

In operation, the solvent to be tested, trichloroethylene, was pipetted into the bulb and the stopcock closed and weighed by suspending it in the analytical balance (by means of a wire hook not shown in the diagram). The stopcock was then inserted in the rubber stopper, suction applied, and the stopcock opened to some fixed position, depending on whether a small or large concentration was desired. A drop of phosphoric acid served as an efficient lubricant.

The vapor-air mixture was drawn through the apparatus for the desired length of time, after which the stopcock was closed, removed, and reweighed while pure air continued to flush residual vapor from the chamber. The difference in weight represented the amount of solvent which was carried over at a practically constant rate.

Since the experiments were conducted at room temperature, the evaporative loss was uniform throughout, amounting to about 1 mg. for each run. Experiments showed that at various openings of the stopcock and at raised or lowered positions of the inlet tube orifice a range of concentrations of from 10 to 500 p. p. m. was easily obtained.

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Systematic Polarographic Metal Analysis

Characteristics of Arsenic, Antimony, Bismuth, Tin, Lead, Cadmium, Zinc, and Copper, in Various Supporting Electrolytes

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The polarographic characteristics in nine supporting electrolytes of the metals listed in the title have been studied, and complete data are presented for their half-wave potentials and diffusion current constants. The utility of diffusion current constants in practical analytical work is discussed; the use of these constants eliminates the need for calibrating each particular dropping electrode with known concentrations of the substances to be determined.

A PROGRAM has been started in this laboratory with the aim of developing systematic schemes of polarographic analysis that can be applied to the qualitative and quantitative analysis of any mixture of the commoner metallic elements. Soon after this program was initiated it became evident that its logical prosecution requires much more specific and quantitative information concerning the polarographic characteristics of the various metals than one can glean from the existing polarographic literature (*6*), and it was therefore decided to obtain such information before attempting to devise specific analytical procedures.

The present paper presents a compilation of the half-wave potentials, diffusion current constants, and other analytically pertinent characteristics of arsenic, antimony, bismuth, tin, lead, cadmium, zinc, and copper, in nine different supporting electrolytes. These metals were studied as a group because they are so commonly associated in nonferrous alloys and other commercial products, and also because, with the exception of zinc, they are usually separated as a group by hydrogen sulfide precipitation in common analytical schemes.

A simplified method of performing quantitative polarographic determinations, which eliminates the necessity of calibrating each dropping electrode with known concentrations of the substance being determined, is also described.

Experimental

A Model XI Heyrovský Polarograph, manufactured by E. H. Sargent and Co., Chicago, was used for recording polarograms according to the technique which has already been adequately described (*6*). The galvanometer of the instrument was calibrated frequently by the usual method (*6*), and with sufficient care so that the sensitivity factor was known with an accuracy of ±0.3 per cent. With the particular polarograph used the galvanometer deflection on the visual scale was 3.5 per cent larger than on the photographic paper for a given current, apparently because of a difference in the lengths of the two light paths, but both deflections were strict linear functions of the current (as required) within the limits of accuracy of the readings.

H-type cells like those described previously (*6, 13*) were used, with either a saturated calomel or mercury–mercurous sulfate reference working anode, and in all cases measurements were made with the cell in a water thermostat at 25.00° C. The importance of reasonably precise temperature control in polarographic measurements has been stressed elsewhere (*6*). Dissolved air was removed from the solutions by nitrogen which had been purified by passage over copper gauze at about 400° C.

The dropping electrode assembly was the same as that described by Lingane and Laitinen (*13*), and the capillary proper consisted of a 9-cm. length of 6-mm. outside diameter Corning "marine barometer tubing" with a uniform internal diameter of approximately 0.03 mm. The *m*-value was determined and checked daily by means of the simple volumetric instrument previously described (*10*). At the time of this writing the *m*-value of this capillary has remained constant at 2.57 ± 0.01 mg. per second at 25° C. for over eight months; this is a good example of the constant behavior that can be expected of such capillaries when they are accorded the simple maintenance care that has been recommended (*6*). The drop time was in the neighborhood of 3.5 seconds, the exact value depending on the nature of the supporting electrolyte and the potential of the dropping electrode. In all cases the exact drop time was determined at the particular potential at which the diffusion current was measured.

Stock solutions of the various metal salts were prepared from reagent quality materials, and when necessary they were standardized by the usual procedures. Particular care was taken in the preparation of the stock solutions of stannous tin to prevent the formation of stannic salt, because in some supporting electrolytes—e. g., dilute hydrochloric acid—stannic tin contributes to the diffusion current and hence its presence would lead to too high a value for the diffusion current constant of the stannous tin. A stock solution of stannous perchlorate in 1 *N* perchloric acid was prepared by dissolving pure tin in the acid solution in contact with a piece of platinized platinum wire to decrease hydrogen overvoltage; the solution was stored under a nitrogen atmosphere

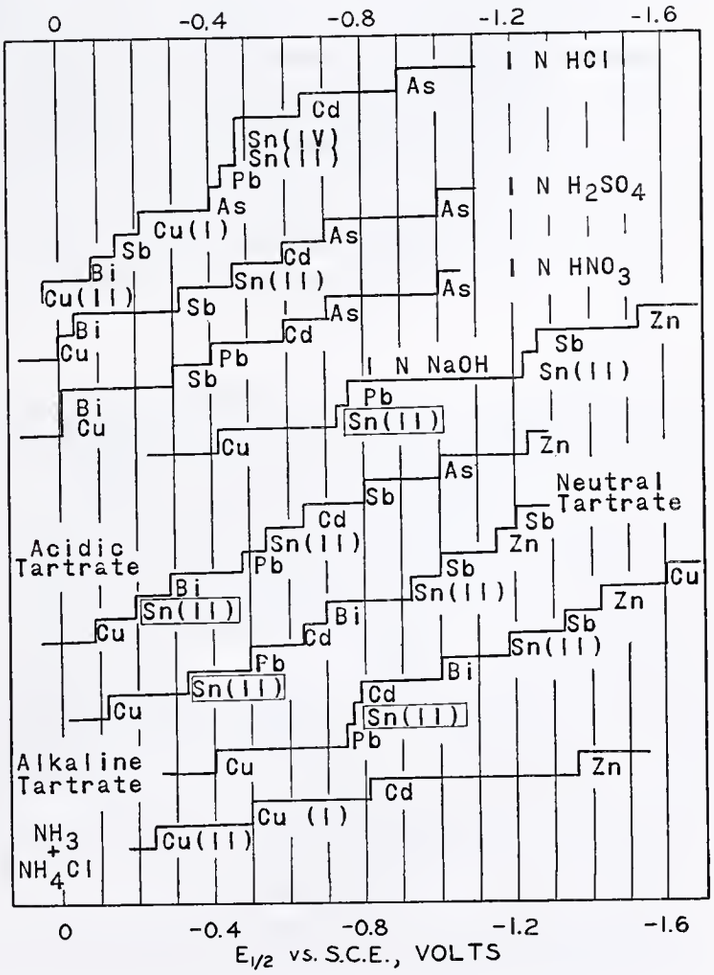

FIGURE 1. CHART OF HALF-WAVE POTENTIALS

TABLE I. HALF-WAVE POTENTIALS

(Values listed are in volts *vs.* the saturated calomel electrode at 25° C. 0.01% gelatin is present in all cases.)

Supporting Electrolyte	As	Bi	Sb	Sn(II)	Sn(IV)	Pb	Cd	Zn	Cu
0.1 *N* KCl	...	Insoluble	Insoluble	Insoluble	Insoluble	−0.40	−0.60	−1.00	+0.04
1 *N* HCl	−0.4 −0.9	−0.09	−0.15	−0.47	−0.47	−0.43	−0.64	[a]	+0.04 −0.22
1 *N* H_2SO_4	−0.7 −1.0	−0.04	−0.32	−0.46	Insoluble	Insoluble	−0.59	[a]	−0.00
1 *N* HNO_3	−0.7 −1.0	−0.01	−0.30	−0.44	Insoluble	−0.40	−0.59	[a]	−0.01
1 *N* NaOH	N. R.[b]	Insoluble	−1.26	−0.73[c] −1.22	N. R.[b]	−0.76	Insoluble	−1.53	−0.42
Acidic tartrate	−1.0	−0.29	−0.8	−0.20[c] −0.54	N. R.[b]	−0.48	−0.64	−1.23	−0.09
0.5 *M* sodium tartrate	N. R.[b]	−0.7	−1.0 −1.2	−0.33[c] −0.92	N. R.[b]	−0.50	−0.64	−1.15	−0.12
Alkaline tartrate	N. R.[b]	−1.0	−1.32	−0.77[c] −1.18	N. R.[b]	−0.75	−0.79	−1.42	−0.4[d] −1.6
1 *N* NH_4Cl + 1 *M* NH_3	...	Insoluble	Insoluble	Insoluble	Insoluble	Insoluble	−0.81	−1.36	−0.24 −0.50

[a] Wave masked by final current increase.
[b] Not reduced at the dropping electrode.
[c] Anodic wave.
[d] See note under copper.

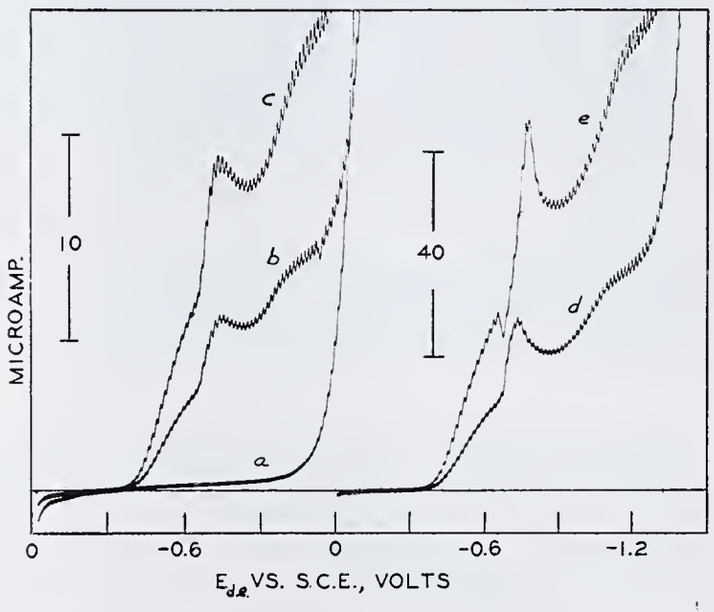

FIGURE 2. POLAROGRAMS OF ARSENIOUS ACID IN 1 *N* HYDROCHLORIC ACID IN THE PRESENCE OF 0.01 PER CENT GELATIN

Concentrations of As_2O_3: (*a*) 0, (*b*) 0.196, (*c*) 0.385, (*d*) 0.742, and (*e*) 1.53 millimolar

in contact with excess metallic tin, and it was standardized by titration against a ceric solution at each time of use by the procedure already described (*11*). In all cases the concentrations were known with an accuracy of ±0.3 per cent or better.

The supporting electrolyte solutions were also prepared from reagent quality materials, and in each case the purity of the solution was checked by running a residual current curve. The concentrations of the components of the supporting electrolytes were known to within ±10 per cent, which is a sufficiently rigorous tolerance as long as the concentration is large compared to that of the substance being determined.

The compositions of most of the supporting electrolytes are clearly shown in Tables I and II. The "acidic tartrate" solution (pH = *ca.* 4.5) comprised 0.4 *M* sodium tartrate and 0.1 *M* sodium hydrogen tartrate, and the "alkaline tartrate" solution (pH = *ca.* 13.0) was composed of 0.5 *M* sodium tartrate and 0.1 *N* sodium hydroxide.

Gelatin, at a concentration of 0.01 per cent, was present in all cases as a maximum suppressor. A 0.2 per cent stock solution was used, and it was preserved effectively against mold growth and bacterial decomposition by addition of a few drops of toluene per 100 cc.

The residual current of each supporting electrolyte was determined and proper correction for it was applied in evaluating the diffusion currents (*6*).

Half-Wave Potentials and General Wave Characteristics

Table I contains a summary of the half-wave potential data that have been gathered in this study, the values being referred to the saturated calomel electrode at 25° C. The theoretical significance and practical utility for purposes of qualitative analysis of data of this kind have been discussed in detail elsewhere (*6*, *9*).

The half-wave potential data of Table I are represented graphically in Figure 1, which is styled in accordance with V. Majer's "polarographic spectrum" (*3*). This chart will be found more convenient than a tabular listing of data for purposes of orientation in planning analytical procedures. For example, suppose an alloy of antimony and lead is to be analyzed. A glance at Figure 1 shows that sodium hydroxide or alkaline tartrate would be the most suitable supporting electrolytes if antimony predominates, because in these media the lead wave precedes that of the antimony, but if lead predominates a hydrochloric acid medium should be used, so that the antimony wave will precede that of the lead.

The characteristics of the waves that are of analytical importance are discussed in the following paragraphs. These brief remarks are limited chiefly to new facts discovered in this investigation, and will serve to supplement previous descriptions (*6*).

ARSENIC. When present in the +5 state arsenic is not reduced at the dropping electrode under any conditions that have been tried to date.

Bayerle (*2*), Kacirkova (*5*), and Bambach (*1*) have shown that +3 arsenic produces complicated polarograms from strongly acid media. Some typical polarograms of arsenious

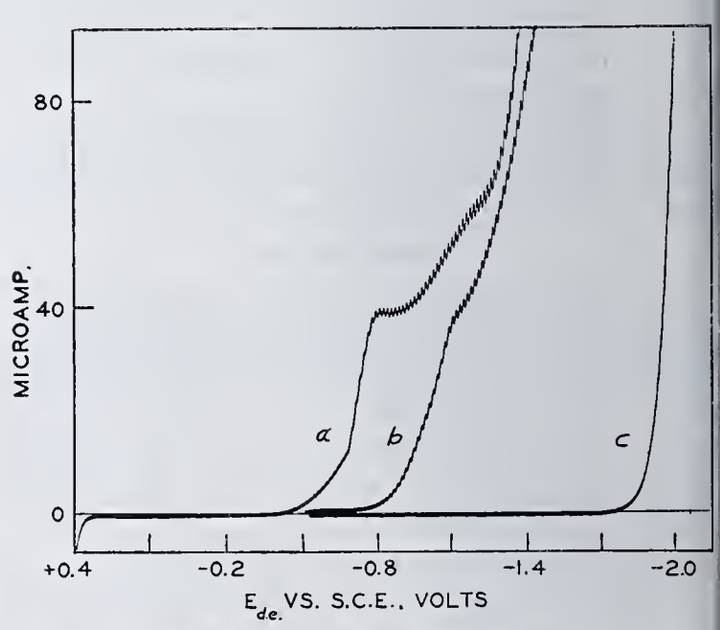

FIGURE 3. POLAROGRAMS OF ARSENIC

In (*a*) 1 *N* sulfuric acid, (*b*) 0.4 *M* sodium tartrate plus 0.1 *M* sodium hydrogen tartrate (pH = 4.5), and (*c*) 1 *N* sodium hydroxide. Concentration of +3 arsenic was 2.00 millimolar in each case, and 0.01% gel was present as a maximum suppressor.

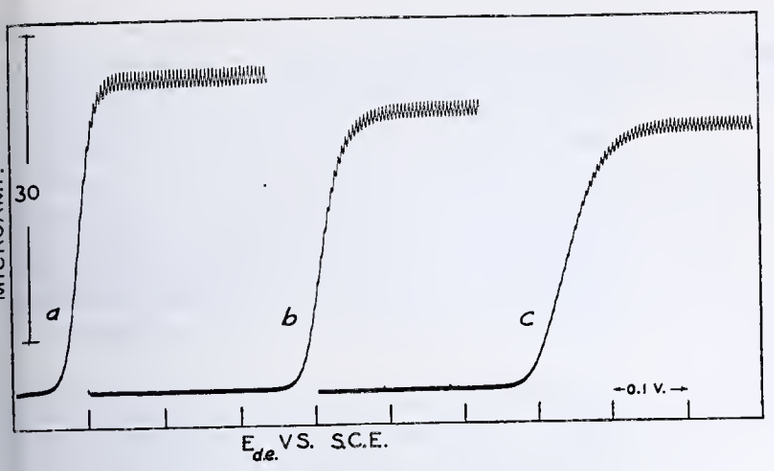

FIGURE 4. POLAROGRAMS OF 2.52 MILLIMOLAR BISMUTH

In (*a*) 1 *N* hydrochloric acid, (*b*) 1 *N* nitric acid, and (*c*) 1 *N* sulfuric acid, in the presence of 0.01% gelatin. Curve *a* starts at 0 volt, and curves *b* and *c* start at +0.3 volt; each voltage mark corresponds to 0.1 volt.

cid obtained with 1 *N* hydrochloric acid as supporting lectrolyte, and in the presence of 0.01 per cent gelatin, are hown in Figure 2.

The arsenic wave is seen to consist of two main parts with some emblance of a diffusion current plateau at −0.8 to −0.9 volt. The wave height at −0.8 volt is directly proportional to the con-entration of arsenious acid, and the diffusion current constant, $_d/(Cm^{2/3}t^{1/6})$, is equal to 8.6 at 25° C. This value is almost twice s large as the corresponding values for antimony and bismuth compare Table II), but since it is to be expected that the diffu-ion coefficient of the uncharged arsenious acid molecule will be arger than the diffusion coefficients of the chloro complex ions of ntimony and bismuth, it seems reasonable to conclude that this vave corresponds to reduction of arsenious acid to the elemental tate rather than to arsine. The poorly defined wave that tarts at about −0.9 volt is probably due to reduction to arsine, s Kacirkova concluded; the height of the wave indicates that his reduction does not proceed completely.

Curve *a* in Figure 3 is a typical polarogram of arsenious acid in).5 *M* sulfuric acid, and its character is similar to that obtained rom hydrochloric acid. The development of the diffusion cur-ent is somewhat better than in hydrochloric acid, and it is linearly roportional to the concentration of arsenic. The wave obtained vith 1 *N* nitric acid as a supporting electrolyte is virtually identi-al in all respects with that obtained from dilute sulfuric acid. The data in Table II indicate that the diffusion cur-ent constants in 1 *N* sulfuric and nitric acids are ractically identical with that in 1 *N* hydrochloric cid.

Curve *c* in Figure 3 demonstrates that arsenite on in 1 *N* sodium hydroxide is not reduced at the lropping electrode.

Arsenite ion is not reduced from neutral or lkaline tartrate media, but a poorly developed vave is produced from an acidic tartrate support-ng electrolyte, as shown by curve *b* in Figure 3. It is obvious that this wave is not suitable for nalytical purposes.

BISMUTH. In agreement with the results of Bayerle (*2*), Kacirkova (*5*), and Page and Robinson (*15*), the author found that the +3 bismuth wave is excellently defined in 1 *N* hydro-chloric, nitric, or sulfuric acids, in the pres-ence of 0.01 per cent gelatin as a maximum sup-pressor, as shown in Figure 4.

In 1 *N* hydrochloric acid $E_{3/4} - E_{1/4}$ was found to be 0.022 volt, compared to the theoretical value 0.019 volt (*6*). In 1 *N* nitric acid $E_{3/4} - E_{1/4}$ was equal to 0.026 volt, and in 1 *N* sulfuric acid it was equal to 0.050 volt. It follows from these data that the reduction of $BiCl_4^-$ from 1 *N* hydro-chloric acid proceeds reversibly, but the reduction of BiO^+ from nitric or sulfuric acids is somewhat irreversible. However, all three supporting elec-trolytes are suitable for the determination of bis-muth alone. In agreement with the conclusions of Page and Robinson (*15*) it was found that 1 *N* sulfuric acid is the best of these three supporting electrolytes for de-termination of bismuth in the presence of antimony (compare half-wave potential data in Table I).

Typical bismuth waves in acidic, neutral (pH = 8.8), and alkaline tartrate media are shown in Figure 5.

The bismuth wave is well developed only in an acidic tartrate medium (pH smaller than about 7), and in neutral or alkaline media (curves *b* and *c*) the waves are practically useless for quantitative purposes. Suchy (*17*) has stressed the use of a "neutral" tartrate supporting electrolyte, but since he added solutions of bismuth nitrate, which must have contained some free nitric acid, to a sodium tartrate solution, the solu-tions that he investigated must have actually had a pH value considerably smaller than that of a pure sodium tartrate solution. In the present experiments with "neutral tartrate" media the solutions were neutralized with sodium hydroxide to the pH of pure 0.5 *M* sodium tartrate (8.8 to 9.0) after the bismuth solution was added.

ANTIMONY. Antimony in the +5 state, like the cor-responding compounds of arsenic, is not reduced at the drop-ping electrode from any of the supporting electrolytes so far studied.

Typical polarograms of +3 antimony in 1 *N* hydrochloric and 1 *N* nitric acids are shown in Figure 6 (see also Page and Robinson, *15*). Both media are suitable for the determina-tion of antimony in the absence of interfering substances, and, except for the peculiar irregularity near the top of the wave in hydrochloric acid, the wave forms are normal with excellent diffusion current plateaus.

The occurrence of the irregularity near the top of the wave (curve *a*, Figure 6) is dependent on the concentration of gelatin, and it disappears when the concentration of gelatin is increased above 0.01 per cent. It is significant that this ir-regularity was not observed by Page and Robinson (*15*), who used 0.1 per cent gelatin as a maximum suppressor.

The steep slope of the wave in 1 *N* hydrochloric acid corre-sponds closely to a reversible 3-electron reduction (probably of $SbCl_4^-$), but the smaller wave slopes in nitric acid (and in sulfuric acid) indicate that the reduction of SbO^+ is not perfectly revers-ible at the dropping electrode. Hydrochloric acid should be a

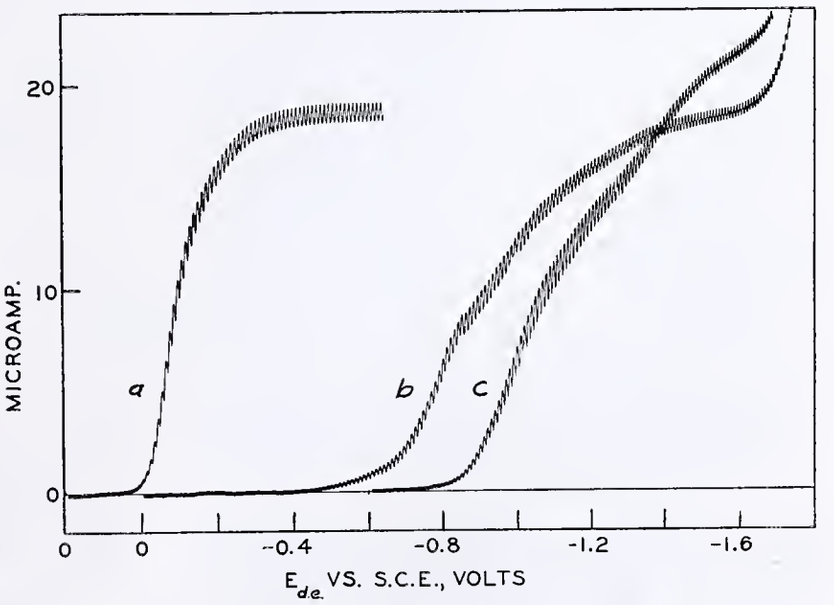

FIGURE 5. POLAROGRAMS OF 2.52 MILLIMOLAR BISMUTH

In (*a*) 0.4 *M* sodium tartrate plus 0.1 *M* sodium hydrogen tartrate (pH = 4.5), (*b*) 0.5 *M* sodium tartrate (pH = 8.8), and (*c*) 0.5 *M* sodium tartrate plus 0.1 *N* sodium hydroxide, in the presence of 0.01% gelatin.

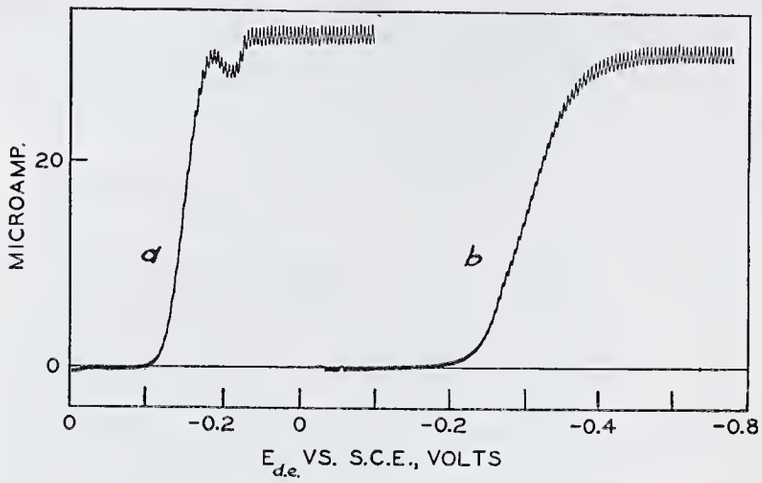

FIGURE 6. POLAROGRAMS OF 2.50 MILLIMOLAR +3 ANTIMONY
In (*a*) 1 *N* hydrochloric acid and (*b*) 1 *N* nitric acid, in the presence of 0.01% gelatin

useful supporting electrolyte for determining antimony in the presence of lead—e. g., in battery plate and other antimonial lead alloys—provided, of course, that interfering elements are either absent or have been removed. Page and Robinson (*15*) have shown that a hydrochloric acid medium is very suitable for determining antimony in various organic antimonial medicinals.

Polarograms of +3 antimony in 1 *N* sulfuric acid and 1 *N* sodium hydroxide are shown in Figure 7; the wave forms and diffusion currents in both media are very satisfactory.

The second small wave at −1.0 volt in 1 *N* sulfuric acid (curve *a*) may be due to partial reduction of the antimony to stibine; the smallness of this wave indicates that this reduction does not take place completely, and its presence precludes the determination in the presence of antimony of other metals whose half-wave potentials in dilute sulfuric acid are more negative than about −0.8 volt.

The polarograms of antimony in tartrate media of various pH values, shown in Figure 8, demonstrate that only in an alkaline tartrate supporting electrolyte containing excess hydroxide ion is the diffusion current sufficiently well defined to be used for exact quantitative purposes (curve *d*), although the wave in acidic tartrate medium (curve *c*) may be useful for semiquantitative work when other conditions preclude the use of an alkaline tartrate solution. The wave in neutral sodium tartrate solution in the absence of gelatin (curve *a*, pH about 9) is seen to comprise three distinct parts. Since reduction to oxidation states between +3 and the metal is highly improbable, this triple wave probably corresponds to the existence of the +3 antimony in three different ionic and/or molecular states in a sodium tartrate solution, with the antimonyl tartrate ion predominating.

STANNOUS TIN. Excellently defined waves are obtained with 1 *N* hydrochloric acid, 1 *N* nitric acid, or 1 *N* sulfuric acid as supporting electrolyte when 0.01 per cent gelatin is present as a maximum suppressor, and as far as polarographic characteristics alone are concerned, all three media are equally suitable for the determination of stannous tin. In 1 *N* hydrochloric acid an indication of an anodic wave, corresponding to the oxidation of tetrachlorostannite ion to hexachlorostannate ion, was observed at about −0.1 volt *vs.* the saturated calomel electrode, but the wave was too poorly developed to be of any analytical use.

Smrz (*16*) claimed that stannite ion in 1 *N* sodiu hydroxide produced two cathodic waves at about −1. and −1.55 volts, and that when a stannite solution wa allowed to stand, the height of the first wave increase while that of the second decreased. He concluded tha the first wave at −1.2 volts was due to the reductio of stannate ion produced by the slow disproportionatio of stannite ion, but this conclusion is erroneous becaus stannate ion is not reduced at the dropping electrod (see below). The author's experience is considerabl at variance with Smrz' results in other respects.

The polarogram of stannite ion in 1 *N* sodium hydroxid comprises a very well developed anodic wave at −0.73 vol and only a single cathodic wave at −1.22 volts, as show in Figure 9. The cathodic and anodic diffusion current are exactly equal, and it is evident that the cathodic wav results from the reduction of stannite ion to the metalli state and the anodic wave corresponds to oxidation of star nite ion to stannate ion. The polarograms in Figure 9, an several others that have been obtained, show no indica tion of the second cathodic wave at −1.55 volts that Smr reported. From the fact that the anodic half-wave po tential is −0.73 volt, whereas the reversible potential of th stannite-stannate couple is −1.21 volts *vs.* the S. C. E., it is clea that the oxidation of stannite ion proceeds irreversibly at the drop ping electrode. The cathodic half-wave potential is several tenth of a volt more negative than one would predict from the reversibl potential of the stannite-tin couple and a reasonable estimate o the free energy of formation of tin amalgam, from which it mus be concluded that the reduction of stannite ion also takes plac irreversibly. This irreversibility of the electrode reactions is o no importance in practical analytical work, since the diffusio currents are so well defined.

Stannite ion in 1 *N* sodium hydroxide is unstable to the exten of about 7.7 kcal. per mole with respect to disproportionation int stannate ion and metallic tin (*8*), and therefore when it is used a a determining form of tin the solution must be freshly prepare and protected carefully from air oxidation. However the autho observed no change in any of the characteristics of the stannit wave after a solution stood for one hour protected from atmos pheric oxygen, and hence with pure solutions the disproportion ation evidently takes place slowly. This may not be true wit impure solutions, or in the presence of metallic tin.

The polarogram of stannite ion in sodium hydroxide medium i very similar to that of stannous tin in an alkaline tartrate solutio (*11*), and the cathodic and anodic half-wave potentials in the tw media are almost identical (compare Table I). Furthermore inspection of the data in Table II shows that the diffusion curren constant in an alkaline tartrate solution (2.86) is about midwa

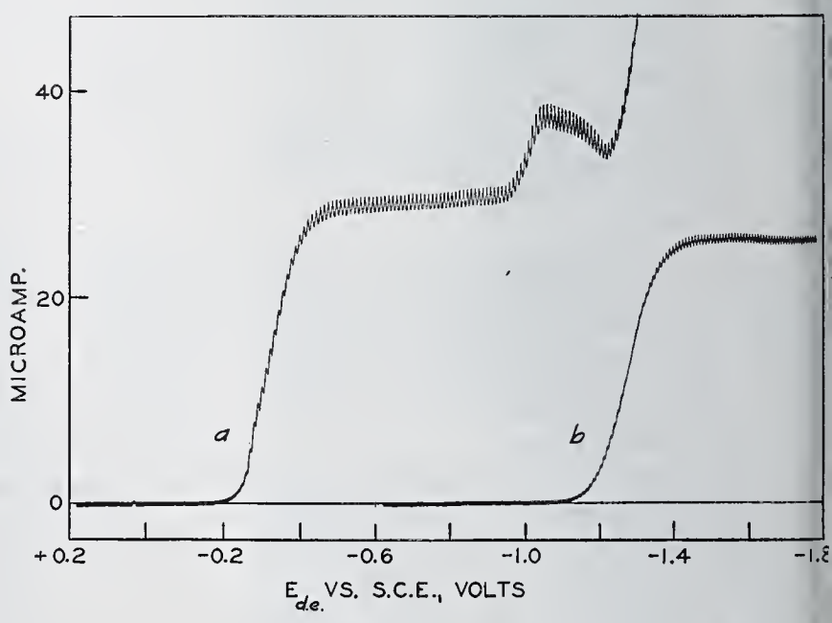

FIGURE 7. POLAROGRAMS OF 2.50 MILLIMOLAR +3 ANTIMONY
In (*a*) 1 *N* sulfuric acid and (*b*) 1 *N* sodium hydroxide, in the presence of 0.01% gelatin

etween the value in an acidic tartrate solution (2.41) and that of annite ion (3.45). It may be concluded that a considerable roportion, although not all, of the stannous tin in an alkaline artrate solution actually exists as stannite ion.

The characteristics of the stannous waves in various tartrate edia have been discussed in a previous paper (*1*).

Stannic Tin. Curve *a* in Figure 10 is a typical polarogram f stannic tin in 1 *N* hydrochloric acid; the character of the ave leaves much to be desired. Since the limiting current s not well developed, but increases uniformly with increasing egative potential, the wave cannot be recommended for xact quantitative work.

The small pre-wave starting at about −0.05 volt, whose height oes not correspond to any integral electron requirement, indiates that in addition to the predominating $SnCl_6^{--}$ ion the soluion also contains stannic tin in another form that is more easily educible. Furthermore, the fact that two separate waves are btained shows that the equilibrium between the two species is stablished very slowly. In view of the well-known tendency of tannic chloride solutions to hydrolyze slowly, even in the presnce of a relatively high concentration of hydrogen ion, it is likely hat the more easily reducible species is a hydrolysis product of ome sort. The half-wave potential of the main stannic wave in hydrochloric acid medium is identical with that of the stannous ave (compare Table I).

Stannic tin in either 1 *N* sulfuric or nitric acids is too reatly hydrolyzed, with the precipitation of basic salts, to ermit the use of these media as supporting electrolytes.

Stannic tin is not reduced from its solutions in sodium ydroxide (curve *b*, Figure 10), nor from any of the three artrate solutions. Although this is rather disappointing rom the standpoint of determining tin itself, it will probably rove to be advantageous in connection with the determinaion of other metals, such as antimony, bismuth, or lead, in the resence of stannic tin.

Lead. The lead waves from 0.1 *N* potassium chloride, *N* hydrochloric or nitric acids, and 1 *N* sodium hydroxide re very well developed with excellently defined diffusion urrents. All these media can be recommended for the etermination of lead.

Normal well developed waves are also obtained from the arious tartrate media, except that in an alkaline tartrate solution the wave requires a fairly great voltage span (*ca.* 0.4 volt) for full development of the diffusion current.

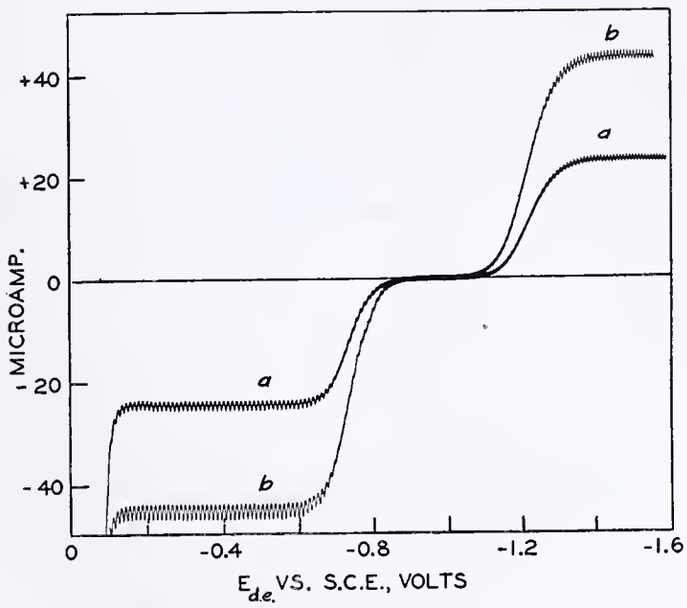

Figure 9. Polarograms of Stannite Ion

In 1 *N* sodium hydroxide in the presence of 0.01% gelatin. Concentrations of tin: (*a*) 3.04, (*b*) 5.59 millimolar.

Cadmium. With either 1 *N* hydrochloric or nitric acid as supporting electrolyte the cadmium wave is excellently defined and both media are suitable for quantitative purposes.

The wave obtained from 1 *N* sulfuric acid can be recommended only for qualitative or rough quantitative measurements because the diffusion current does not reach a perfectly constant value, but increases gradually with increasing negative potential up to the potential of hydrogen discharge. It may be possible to obtain a better diffusion current in a more dilute sulfuric acid solution, but this has not been investigated.

With either an acidic or neutral tartrate supporting electrolyte the wave form is entirely normal with a very good diffusion current plateau, and both media can be recommended for the quantitative determination of cadmium.

On the other hand, an alkaline tartrate supporting electrolyte is not suitable for the determination of cadmium. In such a medium the wave itself is well enough defined, but the limiting current is abnormally small and it is not directly proportional to the concentration of cadmium. For example, in a typical series of experiments the quantity $i_d/(Cm^{2/3}t^{1/6})$ decreased from 1.36 to 0.33 when the cadmium concentration was increased from 1 to 18.6 millimolar. These data show that the limiting current is not diffusion controlled, and hence it has little analytical value.

The wave obtained from an ammoniacal supporting electrolyte is normal in all respects with an excellent diffusion current plateau.

Zinc. Zinc cannot be determined in the presence of a large concentration of hydrogen ion, because the wave is masked by hydrogen evolution.

The reduction of zincate ion from 1 *N* sodium hydroxide produces a well developed wave; the diffusion current is reached just before the discharge of sodium ion.

Well defined waves are also obtained from neutral or alkaline tartrate media. The voltage span of these waves is rather large (*ca.* 0.4 volt), from which it is evident that the reduction of the tartrate zinc complexes is not thermodynamically reversible, but in both cases a satisfactory diffusion current plateau

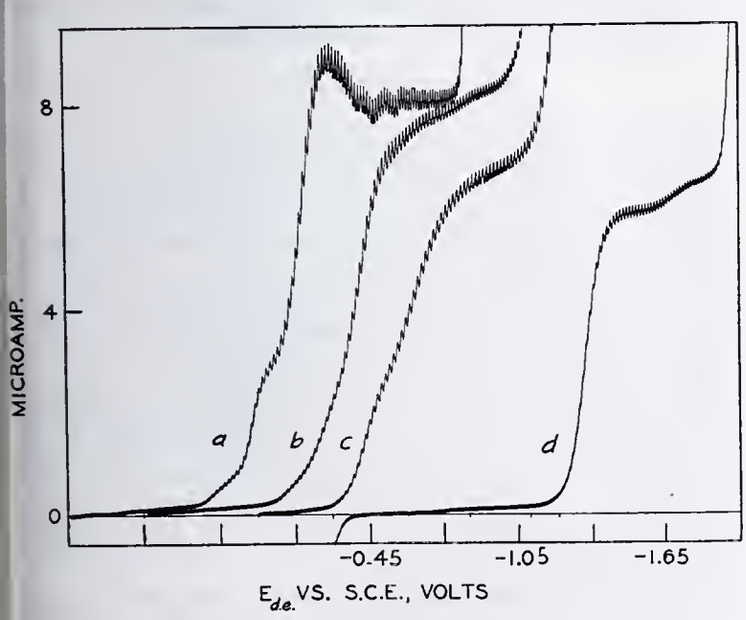

Figure 8. Antimony in Tartrate Media

a. 0.91 millimolar potassium antimonyl tartrate in 0.5 *M* sodium tartrate. *b*. Like *a* except 0.01% gelatin present. *c*. 0.86 millimolar potassium antimonyl tartrate in 0.4 *M* sodium tartrate plus 0.1 *M* sodium hydrogen tartrate plus 0.01% gelatin. *d*. 0.71 millimolar potassium antimonyl tartrate in 0.5 *M* sodium tartrate plus 0.1 *N* sodium hydroxide plus 0.01% gelatin. Each curve starts at −0.3 volt.

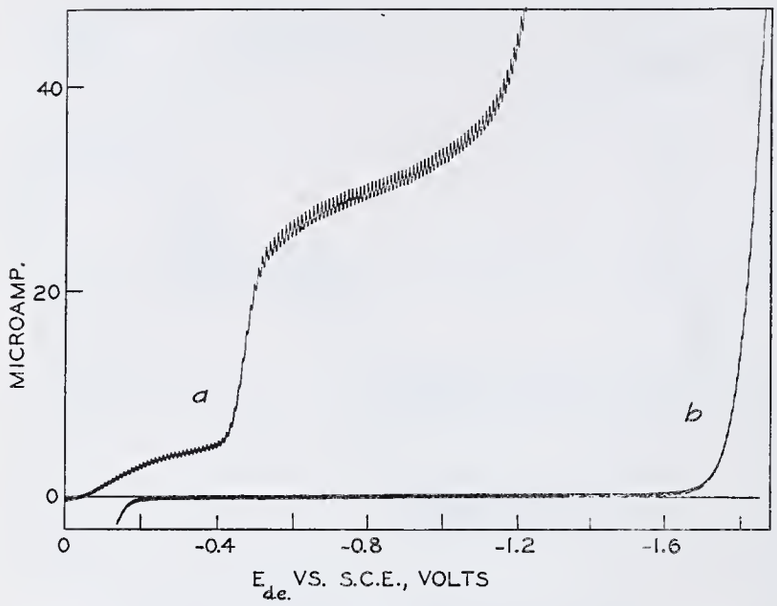

FIGURE 10. POLAROGRAMS OF 2.60 MILLIMOLAR STANNIC CHLORIDE

In (*a*) 1 *N* hydrochloric acid and (*b*) 1 *N* sodium hydroxide, in the presence of 0.01% gelatin

is developed. A distinct wave is also produced in an acidic tartrate medium, but the diffusion current is masked by hydrogen discharge and the wave can be used only for qualitative purposes.

The wave of the tetramminozinc ion from an ammoniacal supporting electrolyte is beautifully developed, and its slope corresponds closely to the value expected for a reversible 2-electron reduction.

COPPER. Typical polarograms of cupric copper in 1 *N* hydrochloric, nitric, and sulfuric acids are shown in Figure 11.

The double wave in a concentrated chloride solution is similar to the characteristic double wave produced in the presence of ammonia, pyridine, and other substances that stabilize the +1 state of copper by complex formation (*6*, *9*). In 1 *N* hydrochloric acid the first wave results from reduction of the +2 copper (probably present as $CuCl_4^{--}$) to the dichlorocuprite ion, $CuCl_2^-$, and the second wave corresponds to further reduction of the dichlorocuprite ion to the metal. The exact half-wave potential of the first stage of the reduction in 1 *N* hydrochloric acid cannot be accurately measured, since it is more positive than the anodic dissolution potential (calomel electrode potential) of mercury, and the wave starts from zero applied e. m. f. From the instability constant of $CuCl_2^-$ (2.9×10^{-6}) quoted by Latimer (*8*) and the standard potential of the cuprous ion–copper amalgam couple (+0.144 volt *vs.* the S. C. E.), and by relations that have already been described (*6*, *9*), the half-wave potential of the second wave should be −0.183 volt *vs.* the S. C. E. for the reversible reduction of $CuCl_2^-$. The observed value (−0.22 volt) agrees sufficiently closely with the theoretical value to indicate that the reduction is reversible at the dropping electrode. From the character of the first wave, and also from a visible amalgam formation, it is evident that +2 copper is reduced by mercury in 1 *N* hydrochloric acid, and hence a separate anode, rather than a quiet pool of mercury as anode, should be used in practical analytical work in order to minimize contact of the solution with mercury. It is usually most convenient to measure the total height of the double wave.

In both 1 *N* nitric and 1 *N* sulfuric acids the reduction of +2 copper produces only a single wave, the reduction proceeding directly to the metallic state. The wave form in 1 *N* nitric acid is normal (curve *b*, Figure 11), but in 1 *N* sulfuric acid a peculiar semblance of a minimum is noted in the diffusion current (curve *c*, Figure 11) and this is followed by a gradually increasing current. Therefore, 1 *N* sulfuric acid cannot be recommended for the quantitative determination of copper, except when it is present at very small concentrations, nor for the determination in the presence of copper of more difficultly reducible substances.

In 1 *N* sodium hydroxide the +2 copper wave is perfectly normal and the diffusion current is very well defined. However, since the solubility of cupric hydroxide in 1 *N* hydroxide ion is small (*ca.* 0.001 *M*), sodium hydroxide possesses only limited usefulness as a supporting electrolyte for the determination of copper in practical analytical work.

The characteristics of the cupric wave in tartrate media have been discussed in a previous paper (*11*). The waves from acidic or neutral tartrate solutions are excellently developed. In an alkaline tartrate solution a small "pre-wave" occurs at about −0.4 volt, and the diffusion current of the main wave at −1.6 volts is masked by the final increase in current from the reduction of sodium ion. It may be possible to eliminate the pre-wave by employing a more alkaline tartrate solution than that used in this study; if this is successful, it will provide a more convenient means than a prior chemical separation of eliminating the interference of copper in the determination of other metals in copper-base alloys.

Diffusion Current Constants and Their Application to Practical Analyses

An ideal polarographic procedure would permit the determination of a substance from a single polarogram, or even from a single measured value of the diffusion current, without the necessity of calibrating the particular dropping electrode that is used with known concentrations of the substance in question. In addition to placing polarographic analysis on a relatively "absolute" basis, such a procedure possesses the important practical advantage of saving the considerable amount of time that must otherwise be spent in preparing standard solutions of each substance to be determined and in obtaining calibration polarograms. Chiefly as a result of the fundamental studies that were made by Ilkovič in Professor Heyrovský's laboratory in Prague (*4*), the factors which govern a polarographic diffusion current are now so well known that the application of this simplified technique to practical analyses is perfectly feasible, when certain fundamental data are available.

It was first demonstrated by Ilkovič (*4*), and later confirmed by others (*6*, *12*), that the diffusion current is expressed completely and quantitatively by the following equation:

$$i_d = knD^{1/2}Cm^{2/3}t^{1/6} \qquad (1)$$

in which i_d is the diffusion current in microamperes, n is the number of electron equivalents per molar unit of the electrode reaction, D is the diffusion coefficient (sq. cm. per second) of the reducible or oxidizable substance, C is its concentration in millimoles per liter, m is the rate of mercury flow from the dropping electrode in mg. per second, and t is the drop time in seconds. The theoretical significance and general practical utility of this relation have been discussed in detail by Kolthoff and Lingane (*6*), and its validity has been definitely established, particularly as regards the linear dependence of i_d on C and on the quantity $m^{2/3}t^{1/6}$. Since k, n, and D are independent of the characteristics of the dropping electrode capillary, the quantity $knD^{1/2}$, which is experimentally determinable as $i_d/(Cm^{2/3}t^{1/6})$, is a fundamental constant for any given substance, and is referred to below as the "diffusion current constant". This term has previously been applied to the ratio i_d/C (*6*), but since this ratio depends on the properties of the dropping electrode capillary—i. e., on $m^{2/3}t^{1/6}$—it is recommended that this usage be discontinued in favor of the more fundamental quantity $i_d/(Cm^{2/3}t^{1/6})$.

According to theoretical considerations (*6*), constant k in Equation 1 should be equal to 605 at temperatures between about 15° and 40° C., when the other quantities are expressed in the units indicated above. The approximate correctness of this value has been verified experimentally in the few cases where sufficiently accurate values of diffusion coefficients are available to permit a comparison (*6*). In most cases, however, it is not possible to employ this theoretical constant to calculate diffusion

TABLE II. DIFFUSION CURRENT CONSTANTS

[Values listed are $i_d/(Cm^{2/3}t^{1/6})$ at 25° C. Values in italics are uncertain because of poorly defined diffusion currents. In all cases 0.01% gelatin was present as a maximum suppressor.]

upporting Electrolyte	As	Bi	Sb	Sn(II)	Sn(IV)	Pb	Cd	Zn	Cu
.1 *N* KCl	...	Insoluble	Insoluble	Insoluble	Insoluble	3.80	3.51	3.42	3.23
N HCl	*8.6*	5.23	5.54	4.07	*4.8*	3.86	3.58	[a]	3.39
.5 *M* H_2SO_4	*8.4*	4.31	4.94	3.54	Insoluble	Insoluble	*2.6*	[a]	*2.12*
N HNO_3	*8.8*	4.64	5.10	4.02	Insoluble	3.67	3.06	[a]	3.24
N NaOH	N. R.[b]	Insoluble	4.54	3.45	N. R.	3.39	Insoluble	3.14	2.91
cidic tartrate	[c]	3.12	*3.4*	2.41	N. R.	2.37	2.34	[a]	2.37
.5 *M* sodium tartrate	N. R.	*3.0*	*3.9*	2.48	N. R.	2.30	2.34	2.30	2.24
lkaline tartrate	N. R.	[c]	3.54	2.86	N. R.	2.39	[d]	2.65	[a]
N NH_4Cl + 1 *M* NH_3	...	Insoluble	Insoluble	Insoluble	Insoluble	Insoluble	3.68	3.99	3.75

[a] Diffusion current masked by final current increase (discharge of hydrogen or sodium ion).
[b] N. R., not reducible.
[c] No definite limiting current.
[d] Diffusion current not well developed, and not proportional to concentration of cadmium.

urrent constants with any degree of accuracy, because diffusion oefficient data under the conditions existing in polarographic neasurements are not to be found in the literature; therefore hese constants must be determined experimentally.

Table II contains a summary of the diffusion current contants of the metals with which the present investigation is oncerned. Except for the values in italics, which are more r less uncertain because of imperfectly developed difusion currents, the values are believed to be generally acurate to ±2 per cent. The values for lead, cadmium, and inc in 0.1 *N* potassium chloride are taken from a previous ompilation (*6*, *12*), and that for copper in the same medium s based on the measurements of Kolthoff and Orlemann (*7*). All other values were determined in the present study.

The data in Table II can be applied directly to the analysis f unknown solutions of these metals in these particular supporting electrolytes. If we represent the diffusion current onstant by *I*, then the concentration in an unknown case vill be given by

$$C = \frac{i_d}{Im^{2/3}t^{1/6}} \qquad (2)$$

In addition to a knowledge of the diffusion current contant, this method requires that the quantity $m^{2/3}t^{1/6}$ be known or the capillary that is used, but the determination of *m* nd *t* by a technique that has already been described (*10*) equires only a very few minutes' time, and obviously it is nuch simpler than the preparation of a standard solution for ach substance.

The reliability of this technique depends primarily on the existence of a linear relation between i_d and $m^{2/3}t^{1/6}$. Basing their opinion on the excellent work of Maas (*14*), Kolthoff and Lingane (*6*) have concluded that this reation is obeyed with an accuracy of at least ±1.5 per cent. From more recent experience he author is convinced that with *m*-values n the usual range from about 1 to 3 mg. per econd, and drop times between about 2 and 4 econds, the accuracy of this relation is as good s the accuracy with which one can measure a iffusion current.

Although *m* is independent of the potential of he dropping electrode and the medium in which the mercury drops form, the drop time, and hence $m^{2/3}t^{1/6}$, changes appreciably with hanging potential and also with the medium (*6*, *7*). Therefore, *m* can be determined with he mercury drops forming in air (*10*), but it is essential that the drop time be determined in the same medium and at the same potential at which the diffusion current is measured (*6*, *7*).

Although the diffusion current constant is independent of the properties of the dropping electrode, it does depend on temperature and on the composition of the supporting electrolyte, because the diffusion coefficient is a function of these factors (*6*). In most cases the temperature coefficient is in the neighborhood of 1.5 per cent per degree, which requires that the temperature be controlled to at least ±0.5°, preferably at 25° C. Usually the composition of a supporting electrolyte is of more importance than its exact concentration, provided that the concentration is relatively large; in many instances the concentration can be varied by as much as a factor of 2, or even more, without seriously changing the diffusion current constant, although this should never be taken for granted without experimental verification. As a general rule the diffusion current constant of a substance is not influenced by the simultaneous reduction or oxidation of other substances at the dropping electrode, and the values listed in Table II are applicable to mixtures of the various metals.

Gelatin, which is commonly used as a maximum suppressor, frequently has a marked influence on the diffusion current constant, and hence its concentration must be controlled carefully and should not be larger than is actually necessary; 0.01 per cent is usually ample.

In addition to their practical analytical value, the data in Table II are also of theoretical interest because they lead directly to values for the diffusion coefficients, and hence constitute a measure of the apparent sizes, of the various ions. The diffusion current constants of cadmium, zinc, and copper are largest in ammoniacal medium, from which it follows that the ammino complex ions of these metals must be smaller, and perhaps also less subject to the restraining effects of interionic attraction, than the complexes present in the other media. The values for lead, cadmium, zinc, and copper in 1 *N* hydrochloric acid are only slightly larger than in 0.1 *N* potassium chloride, and the difference is of the same order of magnitude in each case.

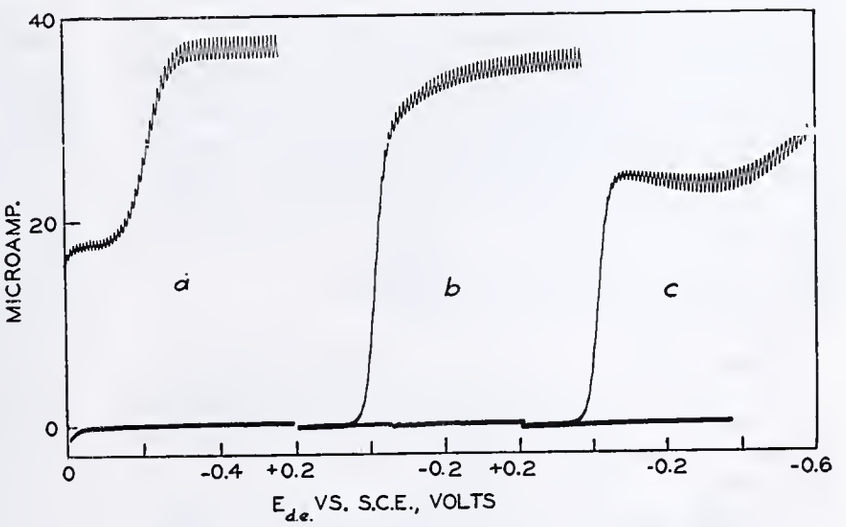

FIGURE 11. POLAROGRAMS OF 4.71 MILLIMOLAR COPPER

In (*a*) 1 *N* hydrochloric acid, (*b*) 1 *N* nitric acid, and (*c*) 1 *N* sulfuric acid, in the presence of 0.01% gelatin

In all cases, except that of copper, the diffusion current constants in the three mineral acids and in sodium hydroxide decrease in the order hydrochloric acid > nitric acid > sulfuric acid > sodium hydroxide, which indicates that the chloro complexes in hydrochloric acid are smaller than the aquo complexes that predominate in nitric and sulfuric acids, and that these in turn are smaller than the hydroxo complex ions present in sodium hydroxide solution.

The values in the tartrate media are smaller than in the other media, which is to be expected in view of the relatively large size of a tartrate complex ion. Furthermore, the diffusion current constants correspond to practically identical values for the diffusion coefficients of the tartrate complexes of the various metals. This leveling effect is doubtless due to the fact that the coordinating tartrate ions are so large compared to the size of the central metal ion, that differences in the size of the central ion, and even in the type and orientation of the bonds, have only a slight influence on the effective sizes of the different complexes.

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An Apparatus for Quantitative Catalytic Hydrogenation

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THE apparatus for catalytic hydrogenation recently described by Noller and Barusch (*3*) is very useful when not more than 50 ml. of hydrogen are to be absorbed; however, it is not suitable for the many occasions when it is necessary to meter with some degree of accuracy as much as several hundred milliliters of hydrogen. The apparatus pictured (Figure 1) fills the entire gap between a strictly micro apparatus and the standard Adams (Burgess-Parr) machine.

This apparatus is a combination, with certain modifications, of features previously reported. The buret system is similar to that described by Fieser and Hershberg (*2*) and the reaction flask together with the stirring device is a slight modification of the one used by Noller and Barusch (*3*). The 50-ml. buret is for small-scale work and the 500-ml. buret is used for larger runs. If more than 500 ml. of hydrogen are to be absorbed, the stirring is slackened or momentarily stopped while the buret is quickly refilled with hydrogen. All the stopcocks are the oblique-bore high-pressure type manufactured by Eck and Krebs, New York, N. Y., who fabricated the glass parts of this apparatus. For those who wish to construct their own apparatus, attention is called to the pressure stopcock recently described by Connelly (*1*). A somewhat less elegant, although reasonably satisfactory, method is to use rubber bands to keep the stopcocks seated firmly. Ordinary stopcocks with none of these precautions will leak hydrogen, especially if extra internal pressure is applied during the hydrogenation as described below.

Flasks of the three sizes indicated afford flexibility in the volume of liquid that can be used, and small coil springs keep the greased ground-glass joint tightly sealed. This joint is reversed to minimize the danger of contamination of the flask contents with grease. A critical dimension is the height of the apparatus, which should not be less than 1 meter, in order that the system may be subjected to either a vacuum or the pressure of an extra atmosphere without forcing mercury out of the manometer.

Some experimenters will prefer using water or the solvent being employed in the hydrogenation as the displacing fluid instead of mercury, especially in view of the current limited availability of mercury. Increased accuracy can probably be obtained if the amount of dead space is reduced by using capillary tubing of about 3-mm. bore in place of the 10-mm. outside diameter standard tubing indicated for the manometer and connections. Unless care is exercised, the stirring may become vigorous enough to drive the stirrer through the wall of the flask, but encasing the iron core in a material such as Saran, instead of glass, may be at least a partial solution of this difficulty.

The air can be displaced from the apparatus by opening the flask stopcock and sweeping with hydrogen; however, unless care has been taken to trap hydrogen in the buret by closing the buret stopcock at the end of the previous hydrogenation, mere sweeping may leave a pocket of air in the buret. It is prudent, therefore, to open the buret stopcock, raise the mercury to a point near the top of the buret, close the screw clamp, and, with the flask stopcock closed, successively evacuate and flood the system with hydrogen three or four times by manipulating the three-way stopcock. Any addition of catalyst or compound after the system has been filled with hydrogen is made by washing the material through the cup into the flask with a little solvent after the internal pressure has been reduced by lowering the mercury in the buret. A small amount of solvent is allowed to remain in the cup, so that air is not admitted to the system. The sequence of addition of catalyst and reagents can be left to the discretion of the investigator; in many cases it is convenient to have the sample in the flask before attaching it to the buret system, but where a highly volatile substance is to be hydrogenated, it is preferable to introduce it through the cup after the sweeping or evacuation procedure has been completed. The catalyst can be reduced in the presence of the sample or the catalyst can be reduced first, a reading

taken, and then the compound added through the cup. The former procedure provides a somewhat more active catalyst, whereas the latter has the advantage of eliminating the necessity of making a blank run to determine the amount of hydrogen absorbed by the catalyst.

Before the initial volumetric reading is taken, the height of the mercury in the buret is adjusted so that the pressure inside the system is equal to the atmospheric pressure, as evidenced by the same mercury level in both arms of the manometer. A facile way of doing this is to raise the level of the mercury in the buret until the internal pressure slightly exceeds the atmospheric pressure and then vent the excess hydrogen to the atmosphere by momentarily opening the three-way stopcock. During the reduction, up to about one atmosphere pressure can be applied by raising the mercury level in the buret and closing the screw clamp, remembering that the internal pressure must be equalized with the atmospheric pressure before reading the volume.

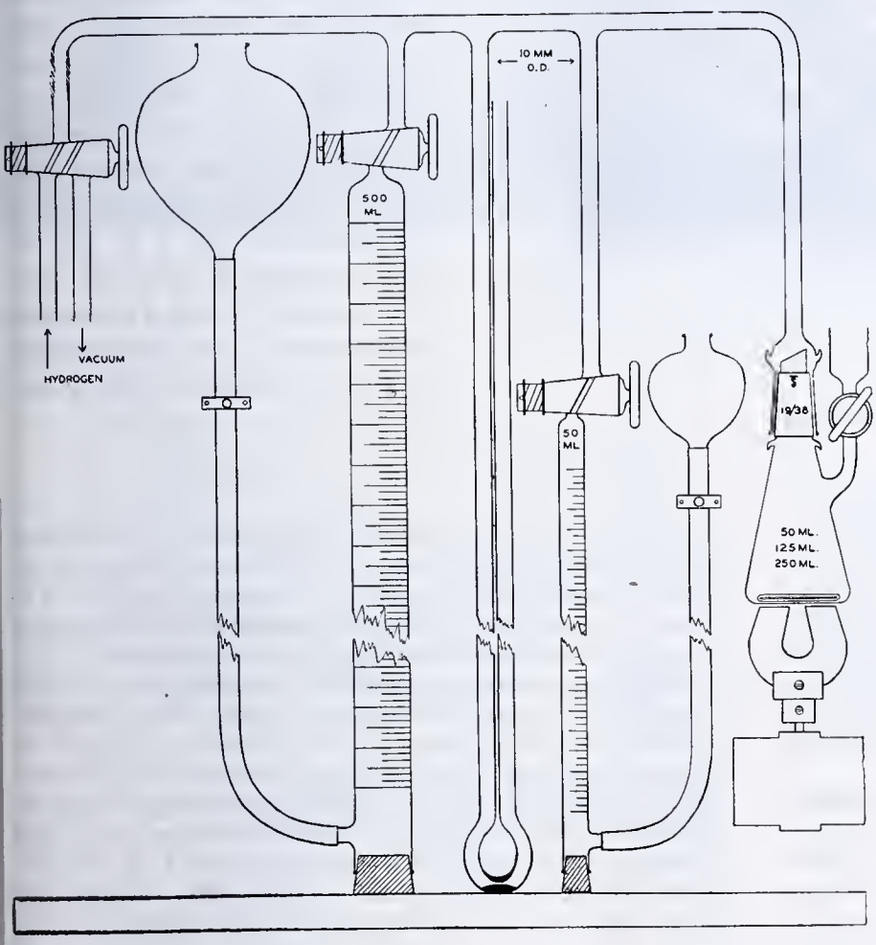

FIGURE 1

The apparatus lends itself very easily to correction for changes in barometric pressure during the experiment, since with the aid of the manometer and leveling bulb a measured differential between the internal and atmospheric pressures can be created at will before making the final volumetric reading. For example, if the barometric pressure increases 5 mm. during the experiment, the mercury in the buret is adjusted so that the level in the right (open) arm of the manometer is 5 mm. below the level in the left (closed) arm before the volume is read. The absolute pressure in the system will then be the same as at the beginning of the experiment.

It is desirable to operate in a constant-temperature room but, in the absence of such a room, temperature changes during the experiment also can be compensated for by proper adjustment of the internal pressure in relation to the atmospheric pressure. If the temperature increases during the experiment, the mercury in the buret should be adjusted so that the internal pressure is greater than the atmospheric pressure before making the final volumetric reading; and, vice versa, if the temperature decreases, the internal pressure should be adjusted so that it is less than the atmospheric pressure. Calculation shows that for this purpose a pressure differential of about 2.5 mm. of mercury corresponds to a change of 1° C. If both the pressure and temperature change, the algebraic sum of both corrections is used in adjusting the manometer levels:

$$\Delta P = P_i - P_f + (T_f - T_i)\, P_i/T_i$$

where ΔP is the increment which the internal pressure should be adjusted in excess of the atmospheric pressure at the end of the experiment—i. e., the number of millimeters the mercury level in the open arm of the manometer must be set above the level in the closed arm. P_i is the initial barometric pressure minus the vapor pressure of the solvent at the initial absolute temperature, T_i; P_f and T_f are the corresponding net pressure and temperature at the end of the experiment. Since these are all known, ΔP is easily calculated. In this way the final volume corrected to the initial temperature and pressure is read directly. The net volume of hydrogen absorbed is then corrected to standard conditions in the usual manner.

Although no rigorous comparison has been made with the rate of hydrogenation obtained when using a shaking device, it can be shown that magnetic stirring affords reasonably rapid reduction. For example, reduction of 1.5 grams of nitrobenzene in ethanol solution was complete (900 ml. of hydrogen) in 1.25 hours although the Adams catalyst used (25 mg.) was over two years old.

Acknowledgment

The collaboration of W. Nudenberg, H. A. Fouche, R. E. Davis, and L. W. Butz of the United States Department of Agriculture is gratefully acknowleged.

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Photoelectric Photometer for Determining Carbon Disulfide in the Atmosphere

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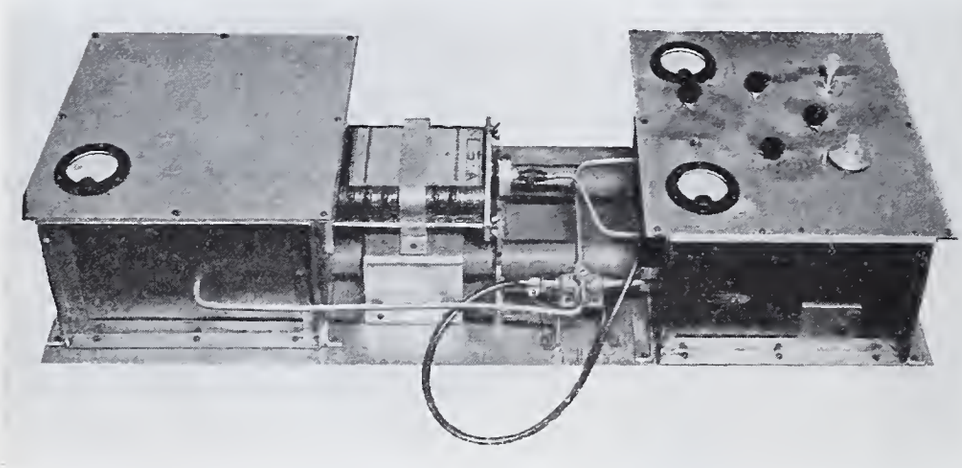

FIGURE 1. PHOTOELECTRIC PHOTOMETER

A photoelectric photometric method has been developed for measuring carbon disulfide in the atmosphere. The range of concentrations covered is 2 to 200 p. p. m. by volume. Time required for an individual determination is 1 minute or less, and the accuracy is 1 p. p. m. The apparatus may be run continuously with a resolving power of 5 to 10 seconds, and an accuracy of 4 p. p. m.

THE problem of developing quick accurate methods for determining concentrations of toxic vapors has been receiving increased attention the past few years. Good analytical methods exist, generally based on colorimetry, for many vapors including carbon disulfide. Although these methods are usually very accurate, the large volume of air required for the sample precludes obtaining anything but an average concentration taken over a time interval that may run as high as 15 minutes or more.

Some of the methods of "grab" sampling for carbon disulfide have been developed to a degree which enables them to be used in continuous recording procedures (*3*, *4*, *5*). These methods have all depended upon some secondary phenomenon, such as color produced by carbon disulfide vapor in solutions of copper acetate and diethylamine in methyl Cellosolve; the conductivity produced in an aqueous solution of sulfur dioxide formed by burning carbon disulfide or the turbidity of a fog produced by first oxidizing the carbon disulfide to sulfur dioxide, and further oxidizing the sulfur dioxide to fog-producing sulfur trioxide. The color produced by the first reaction, the conductivity produced by the second, and the fog produced by the third all permit the use of accurate recording by electric or photoelectric devices. The accuracy remains high, but appreciable lag still exists between time of sampling and actual registry of contamination.

Obviously, a desirable method of recording is one which depends upon some primary property of carbon disulfide; in particular, some property which permits an almost instantaneous quantitative measurement on some form of meter. Such a method has been developed for other organic vapors. The apparatus consists of a simple, sensitive, and rapid ultraviolet photometer which was designed by V. F. Hanson, Electrochemicals Division, du Pont Co. (*2*). The present paper deals with the development of a very similar photom-

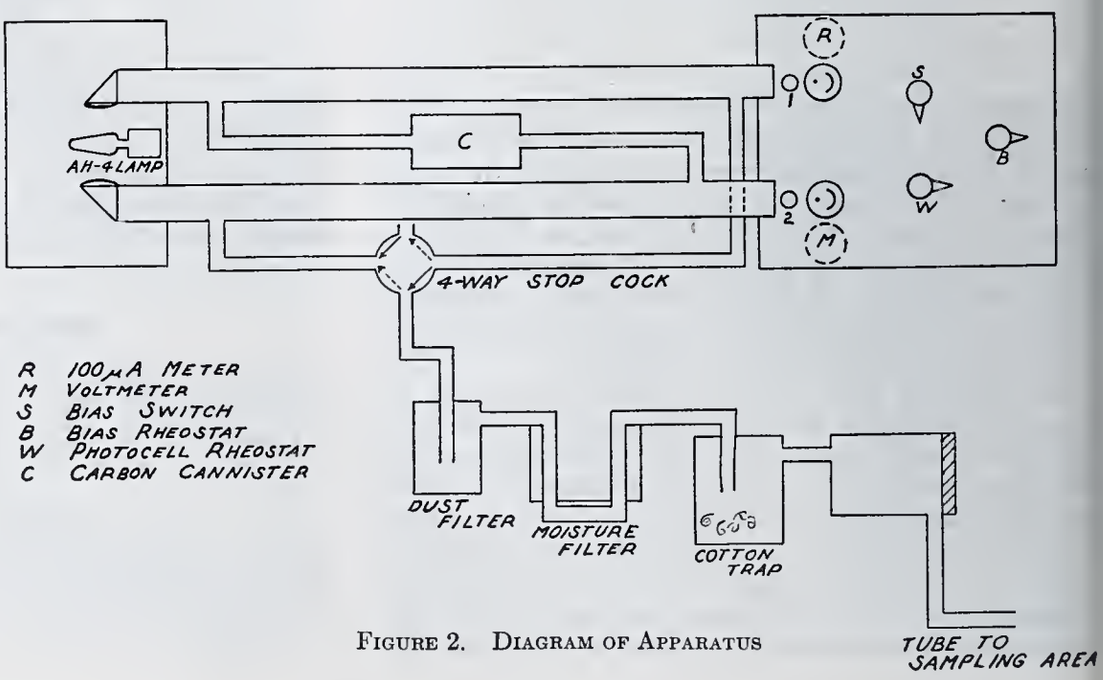

FIGURE 2. DIAGRAM OF APPARATUS

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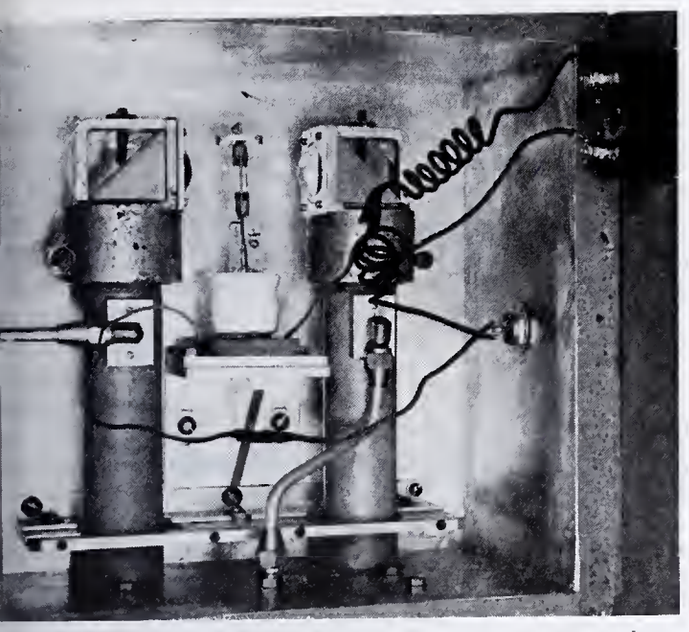

FIGURE 3. LAMP END OF ABSORPTION SYSTEM

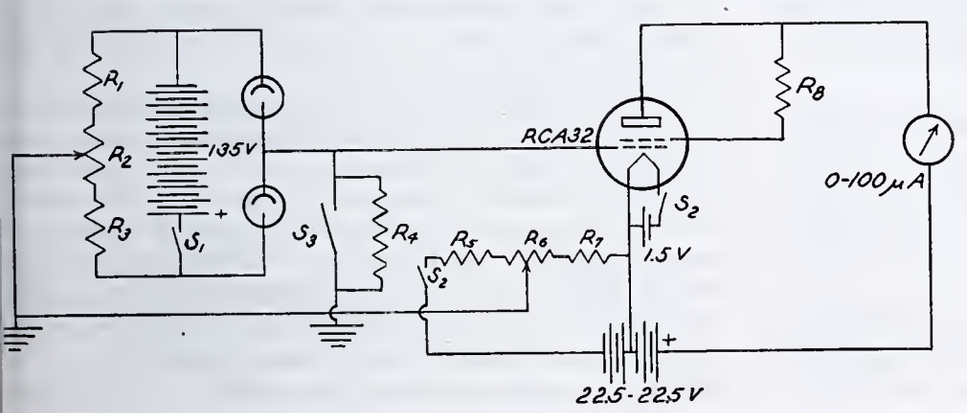

FIGURE 4. ELECTRICAL HOOKUP

R_1, R_3, R_5. 1.0 megohm
R_2. 0.5 megohm
R_4. 2000 megohms
R_6. 250 megohms
R_7. 15 megohms
R_8. 50 megohms

ter, adapted to the specific problem of detecting carbon isulfide.

Optical Basis of the Instrument

Carbon disulfide has an intense absorption in the region of 3100 . Accordingly, an absorption tube was set in front of a monoromator, and with a mercury arc for a light source the absorpon coefficient for carbon disulfide at the mercury line 3132 was etermined. It was found that a column of carbon disulfide apor 1 cm. thick at 1 per cent concentration absorbed 5 per cent. ssuming Beer's law to hold, this indicates that an optical path 0 cm. long would be required for a concentration of 1 p. p. m. to roduce an absorption of 0.02 per cent. The figure of 0.02 per ent was selected because it is the smallest change in light insity that can be detected accurately with the present amplifier.

The absorption of carbon disulfide in this region is a matter f very good fortune, for none of the ordinary constituents f the atmosphere absorbs in this region—for example, Hanon's work on organic solvents showed extremely weak abrption at 3130 Å., although these materials absorbed very rongly at 2536 Å. [Ozone absorbs strongly enough at 3130 Å. be measurable, as shown by Dobson (*1*); but it can be disgarded as a possible contaminant in ordinary processes hich involve carbon disulfide.] Furthermore, none of the ther contaminating vapors from the viscose process, such as ydrogen sulfide, absorbs in this spectral region. Theoretically, then, to determine the amount of carbon disulfide in the air, it is only necessary to set up an absorption tube some 80 cm. long, isolate the 3132 mercury line, and set up an amplifier capable of detecting sufficiently small changes in current.

Instrument Design

The instrument minus power supply is shown in Figure 1. The entire unit is mounted on a pneumatic-tired truck to permit easy transportation to the desired areas.

The apparatus is shown schematically in Figure 2. The light source is a G. E. AH-4 mercury lamp with glass envelope removed. Radiation from the lamp is rendered roughly parallel by condensing lenses of quartz, and reflected down the absorption tubes by totally reflecting prisms of quartz.

The lamp end of the absorption system is shown in Figure 3. The light is received by a pair of matched G. E. FJ-405 sodium photocells. The ends of the absorption tubes are closed with windows of Corex D glass. This glass in combination with the sodium cells gives a system with about 60 per cent of its response due to 3132, 30 per cent due to 3650, 10 per cent due to miscellaneous background, and 0 per cent due to 2536. No absorption has ever been found at 3650 for any samples of air drawn from various parts of the plant areas; accordingly the radiation of this line assumes the character of a constant background, divided equally between the two cells.

The electrical hookup is shown in Figure 4, and is very similar to the amplifier used by Hanson (*2*). The bias circuit is employed to establish a fiduciary zero in the plate circuit. Rough zero setting is obtained by the potentiometer, and accurate balance is obtained by the motion of the micrometer screws; these latter are accurately centered with 0.6-cm. (0.25-inch) extension rods which travel vertically across the faces of the photocells. A vertical motion of 0.025 mm. (0.001 inch) of either of the rods produces a change in light intensity of approximately 0.05 per cent. Micrometer 2 is equipped with a vernier dial to be used in calibrating absorption *vs.* concentration.

In addition to optical symmetry, current and voltage regulation is necessary to obtain the needed photometric accuracy. The constant-current constant-voltage circuit is shown in Figure 5. The voltage is held constant by a Sola 110-volt 125-watt voltage-regulating transformer. The output of this transformer is fed into a resonance network whose characteristics were determined by trial and error after a preliminary rough estimate of the impedances. The present setup holds the current in the AH-4 lamp at about 0.25 ampere; the lamp operates very steadily, and may be expected to have a satisfactory life of some hundreds of hours before any unsteadiness sets in.

Operation of the Analyzer

The apparatus is first adjusted by setting switch *B* to the biasing position, and adjusting the bias rheostat, *S*, for a suitable

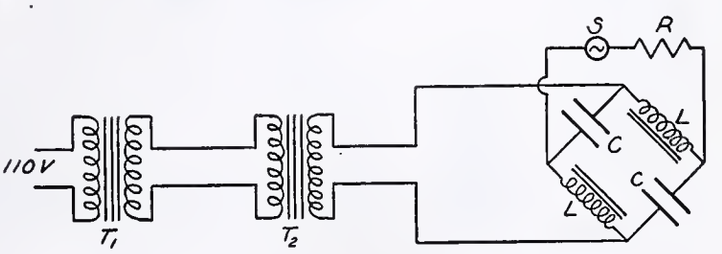

FIGURE 5. CONSTANT-CURRENT CONSTANT-VOLTAGE CIRCUIT

C. 3.5 mfd.
L. 2 henries, 0.5 ampere
R. 1000 ohms, 100 watts
S. G. E. H-4 mercury lamp, envelope removed
T_1. 110-volt, 125-watt Sola voltage regulator
T_2. G. E. H-4 lamp transformer

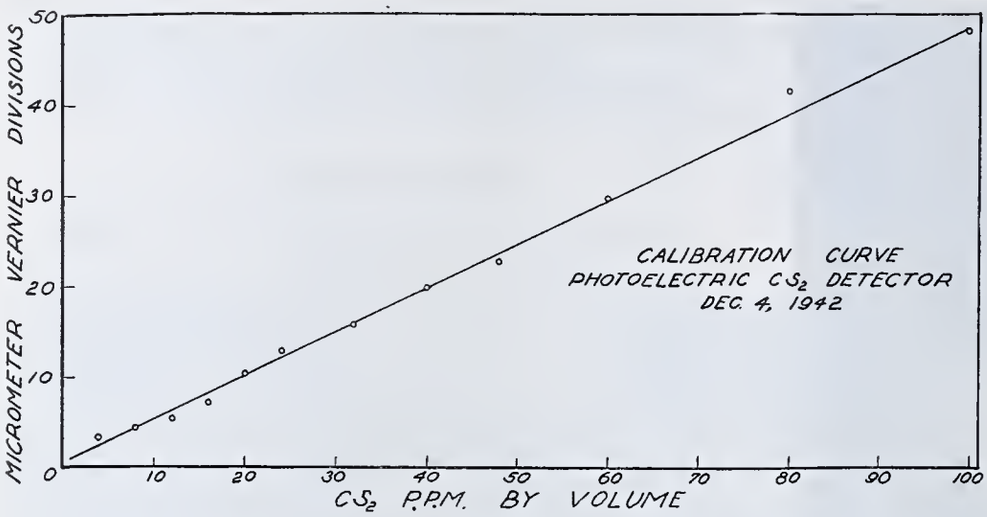

FIGURE 6. TYPICAL CALIBRATION CURVE

plate current reading. *B* is then set to throw the photocells in the circuit; with the balancing rheostat, *W*, set near the middle and with the two micrometer screws set to give shadows about 0.9 cm. (0.375 inch) long on the cell windows, the lamp is adjusted to give about the same plate current reading as the bias. Final adjustment to this fiduciary zero is made with micrometer 1 and the potentiometer.

The air sample is introduced into the apparatus by means of a positive pressure pump. The outlet of the pump leads the air to the following train: (1) a can containing raw cotton to remove traces of pump oil, (2) a moisture trap containing magnesium perchlorate or anhydrous calcium sulfate, and (3) an Alfrax porous filter for removing dust particles. This purification train absorbs no carbon disulfide, and has proved satisfactory over several months' use.

A 4-way stopcock arrangement is provided for passing the sample through one absorption tube, and then through a bed of activated charcoal to the second tube. In this way the instrument always compares transmission through a contaminated column with transmission through an uncontaminated column, and very minute deviations from electrical balance can be detected. As the microammeter in the plate circuit deflects from the fiduciary zero, it is brought back by moving micrometer 2.

The time of reading, together with the time required to flush a new sample through the absorption train, is about 20 seconds. The accuracy is 2 p. p. m., taking the colorimetric method (*3*) as standard. The accuracy may be improved to 1 p. p. m. by using the reversing mechanism provided by the 4-way stopcock, which requires two readings for each determination. Figure 6 shows a typical calibration curve.

For purposes of reading rapidly changing concentrations, the deflection of the microammeter itself may be calibrated, with the micrometer screws being left untouched throughout the run. The microammeter deflection is linear with carbon disulfide concentration up to more than 50 p. p. m., and the accuracy is 4 p. p. m.

The time variation in the carbon disulfide concentration at a fixed station in a purposely poorly ventilated mixing room is shown in Figure 7. The pump was run continuously, and readings were noted at intervals of 10 seconds. The general level at this station is shown at the beginning of the cycle and is around 5 p. p. m. During the run instantaneous readings as high as 50 p. p. m. were noted; the mean was estimated to be about 16 p. p. m. A simultaneous check by the colorimetric chemical method gives values ranging from 14 to 18 p. p. m. Similar checks run at a number of locations show

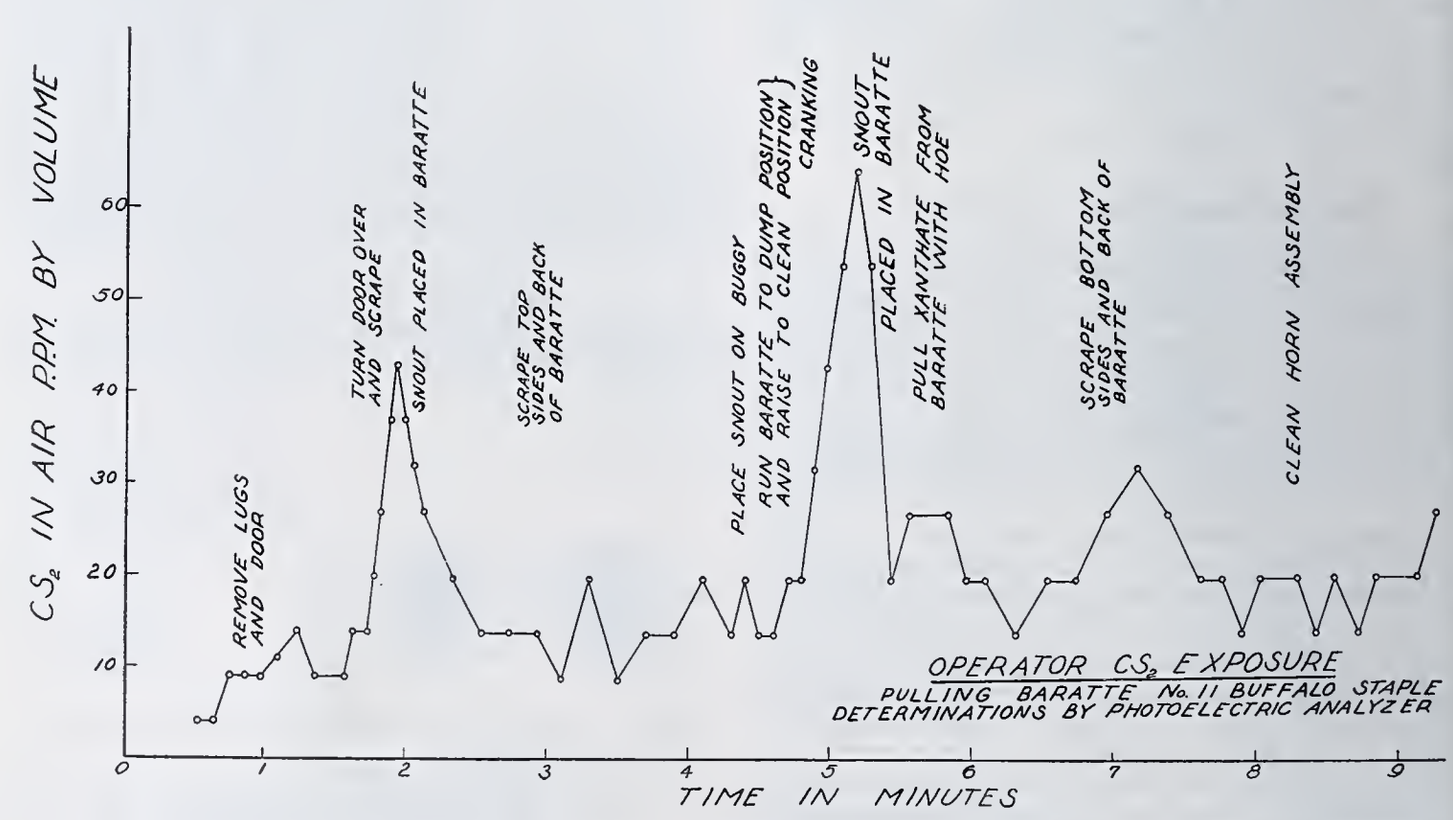

FIGURE 7. CHART OF IMPROPERLY VENTILATED BARATTE

that on the average the discrepancy between the two methods does not exceed 15 per cent for integrated runs.

Acknowledgment

The author wishes to express his appreciation to V. F. Hanson of the Electrochemicals Department, du Pont Co., for many suggestions, to J. W. Ballou and W. H. Warhus, Pioneering Research Section, Technical Division, Rayon Department, for much valuable help, and to other members of the Rayon Department for fostering this research and permission to publish these results.

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Yolk Nomograph for Mayonnaise and Salad Dressing

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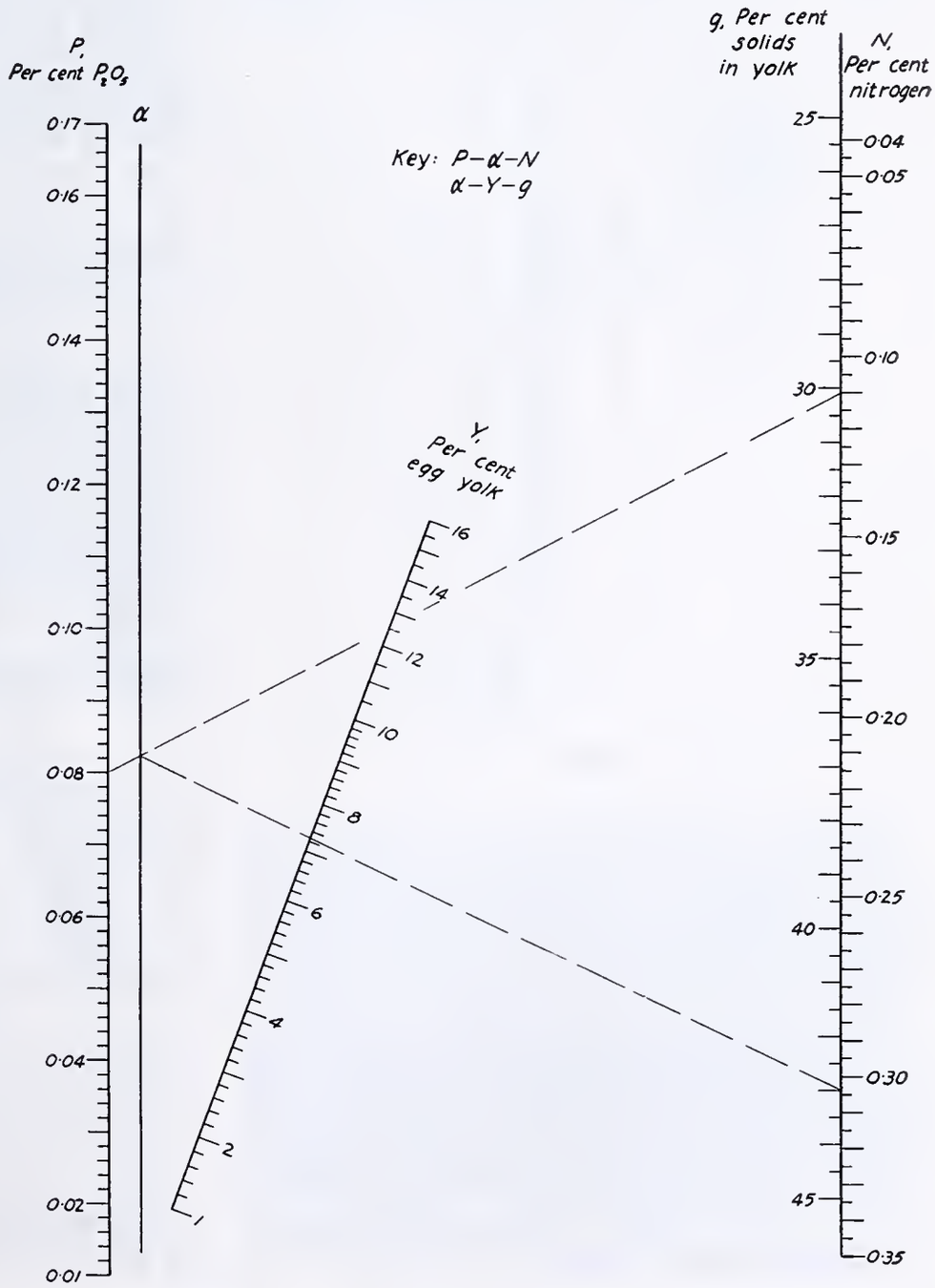
IN CONNECTION with the determination of commercial egg yolk in mayonnaise and salad dressing, Cahn and Epstein (*2*) presented the equation

$$Y = \frac{2899P - 69N}{g - 12.2}$$

where
Y = per cent of commercial yolk
P = per cent phosphorus pentoxide
N = per cent nitrogen
g = per cent solids content of yolk

This equation, consistent with those of the Association of Official Agricultural Chemists (*1*) and based on the analytical data of Mitchell (*3*), can be solved conveniently and accurately by means of the accompanying nomograph. The use of the chart is illustrated as follows:

What is the percentage of commercial egg yolk, containing 43 per cent solids, in mayonnaise which tests 0.08 per cent phosphorus pentoxide and 0.11 per cent nitrogen? Following the key, connect 0.08 on the P scale with 0.11 on the N scale and note the intersection with the α axis. Connect this point with 43 on the g scale and read the percentage of egg yolk on the Y scale as 7.3.



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Improved Steam-Distillation Apparatus

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THE determination of nicotine by the conventional steam-distillation method (*1*) has long been known to have several decided disadvantages. The apparatus is bulky. Because bumping and foaming occur, considerable attention is required during distillation. Since a large volume of distillate must be collected, the time for distillation is rather long.

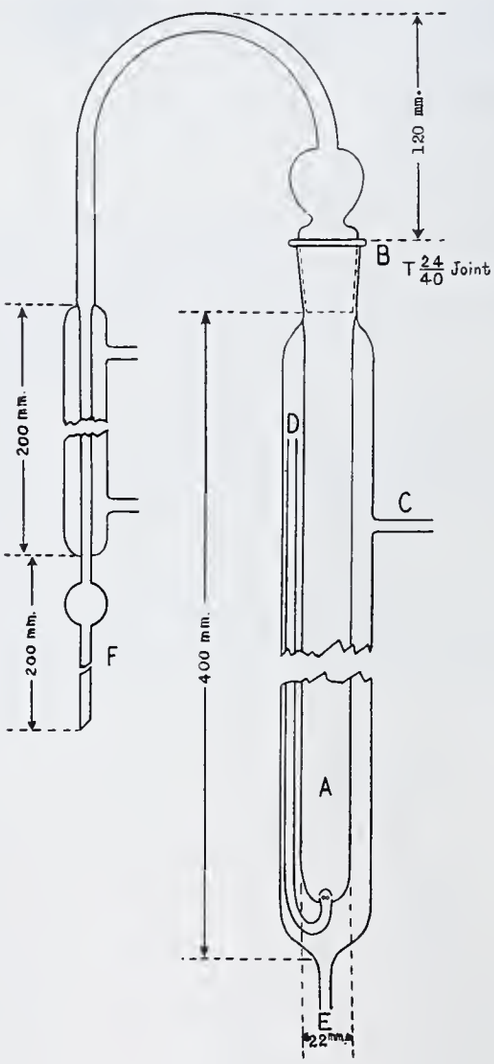

FIGURE 1

Kirk (*2*) has modified the Pozzi-Escot (*3*) apparatus in the construction of micro-Kjeldahl distillation apparatus. An adaptation (Figure 1) of this type of still has proved exceedingly satisfactory for the steam distillation of steam-volatile alkaloids on the macro scale. This modification requires the use of a separate steam generator.

The sample is placed in the inner chamber, *A*, paraffin and alkali are added, and the trap and condenser are connected at the glass joint, *B*, which is lubricated to prevent freezing. The initial volume of the sample and alkali should be as small as practical, preferably less than 25 ml. for an inner chamber with a total volume of 150 ml. Steam is driven in through *C* and passes into the inner chamber through *D*. The drain, *E*, is closed by rubber tubing and a pinchcock, and the distillate is collected at *F*. Where paraffin is added to prevent frothing, some will be carried over into the distillate; but in the case of alkaloids precipitated as silicotungstates and filtered through tared Gooch type crucibles this offers no objection.

This still has many advantages over the conventional ap paratus. The compact construction allows for setting u batteries where large numbers of determinations are to b made. The heating of the distillation chamber by the stean in the outer jacket, together with the entrance of steam a the bottom of the inner chamber, eliminates the possibilit of bumping. The apparatus is easily cleaned by drawing th spent sample into the steam jacket, rinsing well, and allowin the jacket to drain. The narrow inner chamber increase the length of liquid-vapor contact, thereby hastening th distillation. The ground-glass joint at the top of the dis tillation chamber affords a means for introducing the sample alkali, etc., aids in cleaning, and makes the apparatus les rigid, thereby diminishing the danger of breakage. It i possible to take much smaller samples than are ordinaril used for analysis.

The volume of the distillate is dependent on the size o the sample used: 25 mg. of nicotine distilled quantitativel in 100 ml. of distillate and 50 mg. in 150 ml. The accurac of the results obtained with the apparatus is shown in Table I A nicotine solution was steam-distilled and titrated with standard acid and compared with a direct titration of anothe portion of the same size.

The efficiency of the still depends on the time durin which the liquid and steam are in contact. The inner cham ber is heated by the steam held by the outer jacket, whil at the same time the liquid-steam contact is much longe than in the conventional steam-distillation outfit. Thes two conditions much more than compensate for the absenc of flame heating as in the usual apparatus. The efficienc is such that the nicotine may be distilled completely fro a 2-gram sample of tobacco in 0.5 hour.

This apparatus has also been used satisfactorily for th determination of nornicotine and anabasine, both of whic are much less volatile with steam than nicotine. The di mensions given were optimum for the distillation of tobacc samples. Modifications, such as using a larger condense increasing the length or the diameter of the inner chambe or changing the type of trap, may be desirable for some ap plications.

TABLE I. COMPARISON OF STEAM DISTILLATION WITH DIREC TITRATION

(Using 0.02 *N* hydrochloric acid against nicotine)

10 Ml. of Solution		5 Ml. of Solution	
Direct titration	Titration of distillate	Direct titration	Titration of distillate
Ml.	*Ml.*	*Ml.*	*Ml.*
7.58	7.57	3.77	3.78
7.57	7.56	3.78	3.80
7.57	7.57	3.78	3.80
7.58	7.58	3.76	...
Av. 7.575	7.57	3.773	3.793

Acknowledgment

The writers are indebted to O. A. Nelson for the glas blowing involved in making the apparatus described.

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Determination of Mercury in Rubber

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Iodine may be separated from mixtures of mercury and iodine, previous to determination of mercury by the Volhard method, by boiling with nitric acid under controlled conditions. The method has been applied successfully to determination of small amounts of mercury in rubber samples containing iodine.

T HAS recently been necessary to determine small amounts of mercury (0.1 to 5.0 per cent) in natural and synthetic ıbber compounds containing iodine. A survey of the terature showed that the method most often used for the etermination of mercury in organic compounds containing dine involves complete oxidation of the carbonaceous ıaterial by digestion with acid, followed by separation of ıe mercury from the iodine by reduction of the former to the ıetallic state and filtration (*1*, *6*). In searching for a more ɔnvenient method of separation, it was found that this can e accomplished very simply by boiling with nitric acid under ɔntrolled conditions. Free iodine is expelled with but ttle loss of mercury, which can then be titrated with thioyanate. The new method has been applied successfully to ıe determination of mercury in rubber samples containing dine (see Table III).

Apparatus

The apparatus is shown in Figure 1. It consists of a 125-ml. rlenmeyer flask with standard taper 24/40 joint connected to an ir condenser 80 cm. in length and 1 cm. in inside diameter.

Reagents

Standard Mercury Nitrate Solution. Dissolve approxiıately 0.5 gram of pure mercury in 10 ml. of nitric acid in a 1-ter volumetric flask. Add 25 ml. of water and cool to room emperature. Draw off most of the brown fumes and dilute to he mark with water.

Standard Ammonium Thiocyanate Solution (approximately .01 *N*). Dissolve approximately 1.8 grams of ammonium thioyanate in water and dilute to 2 liters. To standardize this olution, pipet 50 ml. of the standard mercury solution into a 25-ml. flask. Add 3 ml. of sulfuric acid, 5 ml. of nitric acid, and).2 gram of potassium sulfate. Color with permanganate and itrate as directed in the procedure.

Ferric Alum Indicator. Dissolve 30 grams of ferric alum [$FeNH_4(SO_4)_2.12H_2O$] in 95 ml. of water and add 5 ml. of colorless ıitric acid.

Procedure

Weigh 20 to 100 mg. of the subdivided rubber sample and ɔlace in a dry 125-ml. Erlenmeyer flask with standard taper oint, taking care not to allow particles of the sample to cling to he walls or neck of the flask. Add about 0.3 gram of potassium ɔersulfate ($K_2S_2O_8$) and tap the flask to collect the rubber partiıles to one side of the bottom of the flask. Add 2 ml. of fuming ulfuric acid and cap tightly with a dry 80-cm. air condenser. If the rubber sample weighs more than 50 mg. it is best to use).5 gram of potassium persulfate and 3 ml. of fuming sulfuric ıcid.) Clamp the neck of the flask to a ring stand and tilt the lask so that the acid covers the rubber sample.

Place in a hood and play a small flame from a Tirrill semimicroburner on the flask below the acid mixture. Heat gently to lecompose the sample, taking great care to prevent loss of iodides ɔf mercury. It is necessary to keep the flame low during the nitial oxidation, so that evolution of white fumes from the tip of the condenser is not too vigorous. As the oxidation proceeds, the lame can be increased, until in the later stages the acid boils and ɔumps. Under no circumstances must the top half of the air ɔondenser be allowed to get even warm. When destruction of the organic matter is complete, remove the flame and allow the flask to cool.

Wash down the walls of the condenser with 15 to 20 ml. of nitric acid (1 to 1). Detach the air condenser and wash the joint with water from a wash bottle. If the sample being analyzed contains less than 5 mg. of mercury, set the condenser aside. Cover the flask with a small watch glass, heat to gentle boiling on a hot plate (do not use a flame), and boil until all the iodine is expelled—i. e., until the solution and vapor are entirely free of color due to iodine and no insoluble iodides of mercury can be seen in the solution. After iodine is expelled, evaporate (rapidly if desired) to about 10 ml. (In case traces of organic matter remain in the solution, evaporate until white fumes begin to be evolved and then add 5 ml. of nitric acid, 1 to 1.) Cool and reserve.

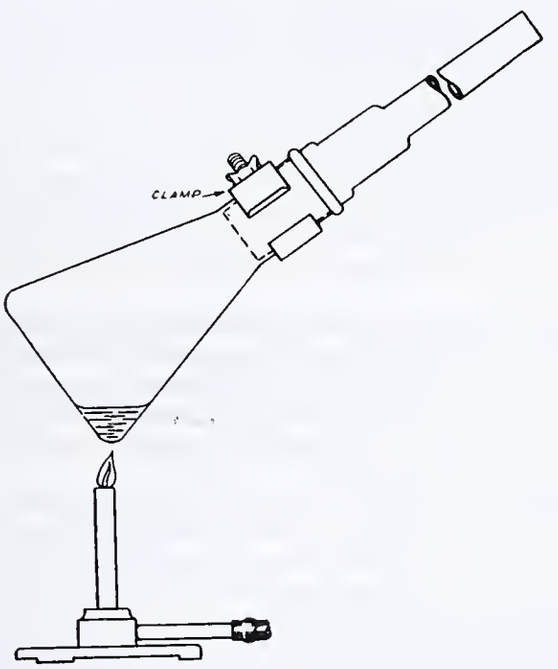

Figure 1. Digestion Apparatus

If the sample contains more than 5 mg. of mercury, replace the air condenser after washing the joint and expel the iodine as directed above, omitting the use of a watch glass. If some iodine collects in the condenser joint during boiling, cool the solution somewhat, break the joint, and allow the iodine to escape. Replace the condenser and continue boiling.

Wash down the sides of the flask (or flask and condenser) with water and remove the condenser if used. Dilute to 30 to 50 ml. with water and cool to room temperature. Add potassium permanganate (2 per cent solution) dropwise until a color is obtained which persists. Dispel the color with a drop or two of 3 per cent hydrogen peroxide. Add 0.5 to 1.0 ml. of ferric alum indicator solution and titrate carefully with 0.01 *N* ammonium thiocyanate solution until the first permanent brownish red color is obtained in the solution. Provide for blank correction:

$$\frac{\text{Ml. of } NH_4CNS \times \text{Hg factor} \times 100}{\text{Sample weight in grams}} = \text{per cent mercury}$$

TABLE I. DETERMINATION OF MERCURY BY THE VOLHARD METHOD

(After expulsion of iodine with nitric acid)

No.	HNO_3 (1:1) Added	Hg Added	Hg Found	Error	Error
	Ml.	*Mg.*	*Mg.*	*Mg.*	%
1[a]	10	1.1	1.1	0.0	..
2	10	3.7	3.7	0.0	..
3	10	5.1	5.1	0.0	..
4[a]	20	5.0	5.1	+0.1	2.0
5	20	5.6	5.6	0.0	..
6	20	5.7	5.6	−0.1	1.8
7	20	10.1	9.8	−0.3	3.0
8	20	12.8	12.4	−0.4	3.1
9	50	11.7	11.3	−0.4	3.4
10	50	19.2	18.8	−0.4	2.1
11	50	70.0	69.0	−1.0	1.4
12	25	100.0	98.0	−2.0	2.0
13	50	93.7	91.0	−2.7	2.9

[a] 200 mg. of K_2SO_4 added with HgI_2.

Discussion

Rubber samples containing mercury and iodine cannot be completely oxidized by treatment with sulfuric acid and 30 per cent hydrogen peroxide or by other mild methods of oxidation. After testing various methods it was found that the oxidation is best accomplished by the Kjeldahl method of Sloviter, McNabb, and Wagner (*5*). In view of the volatility of iodides of mercury and the ease with which they are mechanically carried out of the condenser by the escaping fumes of sulfur dioxide, the rate of digestion must necessarily be slow. Because of this, and in view of the fact that rubber samples containing mercury and iodine are difficult to oxidize, it is best to limit the sample size to 100 mg. or less. Oxidation of certain synthetic rubber samples such as Buna S and Butyl rubber may take several hours, even when the sample size is small. Attempts to decrease the time of oxidation by the use of selenium as a catalyst or by employing the Parr bomb were not successful, as low results were obtained.

With the conditions recommended in the procedure, some loss of mercury occurs during the expulsion of the iodine if the amount of mercury present is greater than about 5 mg. The loss may amount to as much as 2 or 3 per cent in some cases, but for many purposes this accuracy is satisfactory (see Tables I and II).

In preliminary work 30 per cent hydrogen peroxide was used to convert the iodide to free iodine. Very low results were obtained, however, when samples containing large amounts of mercury and iodine were analyzed. This probably resulted from the comparatively low acidity and the decrease in oxidizing strength of the solution as it is boiled. Nitric acid was found to be superior to hydrogen peroxide. The optimum concentration of the acid appeared to be about 1 to 1.

According to Hillebrand and Lundell (*3*) loss of mercury by volatilization as chloride is controlled, to a large extent, by the shape of the vessel and the concentration of the mercury in the solution. The same appears to be true in the present case. Thus, Tables I and II indicate that the loss of mercury increases with an increase in the amount of mercury present and is reduced appreciably by the use of a long reflux tube.

While the actual loss of mercury increases with an increase in the amount of mercury present, the percentage loss first increases and then tends to decrease. The use of the air condenser appears to shift upward the concentration at which maximum per cent error is obtained. Finally, in the expulsion of the iodine, the more vigorous the boiling, the greater the tendency for loss of mercury.

In the determination of mercury in readily oxidizable organic materials, where nitric acid can be used as the oxidant, it may be possible to oxidize the sample and expel the iodine simultaneously. It is also probable that mercury can be separated from chloride or bromide by boiling with nitric acid.

During the development of the proposed method an attempt was made to determine the mercury in the digestion mixture directly by the Rupp method (*4*) without removing the iodine. Preliminary experiments in which known amounts of pure mercuric iodide were analyzed for mercury by the Rupp method showed that low results were obtained when the amount of mercury exceeded about 2 mg. In agreement with Fitzgibbon (*2*) it was found that the addition of gelatin was beneficial. By adding 2 ml. of 1 per cent gelatin solution to the sample before reduction with formaldehyde correct results could be obtained on quantities of mercury up to 5 mg. With larger amounts low results were obtained and a black precipitate which is insoluble in standard iodine solution remained in the bottom of the flask.

When mixtures of rubber and weighed quantities of mercuric iodide (< 5 mg. of mercury) were carried through the complete procedure very low results were obtained. The cause was finally traced to the presence of free iodine in the solution previous to reduction of the mercury with formaldehyde. This iodine was produced in the following manner: After complete oxidation of the rubber the condenser was washed down with 10 ml. of 5 per cent potassium iodide solution; bromine water was added dropwise to oxidize the mercury and sulfur dioxide; when a slight permanent precipitation of iodine occurred the flask was stoppered and shaken to oxidize all the sulfur dioxide; and after 5 minutes the analysis for mercury by the Rupp method was made.

TABLE II. DETERMINATION OF MERCURY BY THE VOLHARD METHOD

(After expulsion of iodine with nitric acid, air condenser used)

No.	Hg Added	Hg Found	Error	Error
	Mg.	*Mg.*	*Mg.*	%
1	0.7	0.7	0.0	..
2	2.7	2.7	0.0	..
3	9.2	9.1	−0.1	1.1
4	13.4	13.3	−0.1	0.7
5	31.6	30.9	−0.7	2.2
6	53.8	52.7	−1.1	2.0
7	118.2	116.6	−1.6	1.4

Theoretically, free iodine should cause no trouble in the reduction of the mercury with formaldehyde, since the hypoiodite is reduced to iodide. Actually, however, when iodine is present the mercury is precipitated in a form that does not dissolve completely in standard iodine solution. Black specks of undissolved material and cloudy solutions are encountered and the results are very low.

Attempts to improve the results by titration of the free iodine in acid solution with sulfite or thiosulfate failed, as did also reduction of the iodine with hydrogen peroxide in alkaline solution. It was therefore concluded that the Rupp method could not be used for direct analysis of the mercury in the digestion mixtures.

TABLE III. DETERMINATION OF MERCURY IN RUBBER–MERCURIC IODIDE MIXTURES

No.	Hg Added	Hg Found	Error
	Mg.	*Mg.*	*Mg.*
1	0.1	0.1	0.0
2	0.5	0.5	0.0
3	0.7	0.7	0.0
4	1.9	1.9	0.0
5[a]	3.0	3.0	0.0
6[b]	4.2	4.2	0.0
7[c]	4.6	4.6	0.0
8	6.2	6.1	−0.1
9[d]	6.4	6.2	−0.2
10[e]	0.5	0.5	0.0
11[e]	2.5	2.5	0.0
12[e]	5.6	5.6	0.0
13[e]	5.8	5.7	−0.1

[a] 25 mg. of KI added with HgI_2.
[b] 100 mg. of rubber, 0.5 gram of $K_2S_2O_8$, and 3 ml. of fuming H_2SO_4 used.
[c] 0.5 gram of $K_2S_2O_8$ and 3 ml. of fuming H_2SO_4 used.
[d] 50 mg. of KI added with HgI_2.
[e] Mercury determined by Rupp method.

The only satisfactory procedure found was that in which the iodine was expelled by boiling with nitric acid, followed by complete expulsion of all nitrates, and finally, addition of potassium iodide, and analysis by the Rupp method in the usual way employing gelatin. This method has been shown to be satisfactory for small amounts of mercury (see Table III), but because of its limited applicability and its complexity it does not compare with the Volhard method.

Experimental

Weighed quantities of pure mercuric iodide together with 3 ml. of sulfuric acid and various quantities of nitric acid (1 to 1) were placed in 125-ml. Erlenmeyer flasks. The iodine was then expelled and the mercury determined by the Volhard method as directed in the procedure, without use of the air condenser. Results are recorded in Table I.

The experiment was repeated using an 80-cm. air condenser. The mercuric iodide was treated with 3 ml. of sulfuric acid, 15 ml. of nitric acid (1 to 1), and 0.2 gram of potassium sulfate. The results are recorded in Table II.

Weighed quantities of pure mercuric iodide and 20 to 30 mg. of a mercury-free gum rubber stock were placed in 125-ml. Erlenmeyer flasks. The samples were then treated as directed in the procedure, using 0.3 gram of potassium persulfate and 2 ml. of fuming sulfuric acid. No air condenser was employed in the expulsion of the iodine. The results are recorded in Table III.

Summary

A very simple method has been developed for the separation of iodine from mixtures of mercury and iodine previous to the determination of the mercury by the Volhard method. The method has been successfully applied to the determination of small amounts of mercury in rubber samples containing iodine. The Rupp method has been investigated.

Acknowledgment

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Benzoin as Fluorescent Qualitative Reagent for Zinc

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SEVERAL reagents which give fluorescent compounds with zinc ions have been recorded in the literature. Lutz (*3*) has shown that urobilin and sterobilin, products of body metabolism, give a green fluorescence with zinc. However, cadmium, calcium, cobalt, copper, magnesium, nickel, potassium, and sodium give a similar fluorescence. The zinc precipitate with 8-hydroxyquinoline gives a yellow fluorescence but identical results can be obtained with other elements that precipitate with this reagent. The dyestuffs morin and cochineal (*2*) both fluoresce in the presence of zinc as they do with many other ions. Textbooks in qualitative analysis generally recommend dithizone, potassium ferricyanide with diethylaniline or Orange IV, mercury thiocyanate, or the Rinman's green test as confirmatory for zinc. Difficulty with these reagents is encountered due to lack of specificity, sensitivity, or indefinite results.

Yoe and Sarver (*5*) list some forty organic compounds that may be used as reagents for zinc. Of these the ones mentioned above are most widely used in qualitative analysis. Organic reagents which coordinate with the metallic ions often have a hydroxyl and a carbonyl group adjacent to or near one another. In testing compounds of this type for fluorescent reactions with the metallic ions it has been found that benzoin, $C_6H_5.CHOH.CO.C_6H_5$, serves as an excellent qualitative reagent for zinc.

Characteristics of the Reagent

Since benzoin is commonly made as a student preparation in elementary organic chemistry laboratories, it is readily available in large quantities. Benzoin is moderately soluble in 95 per cent alcohol and dissolves easily on warming. This solution has a faint green fluorescence but when diluted in the test it offers no interference. It was found during the course of these experiments that a freshly prepared solution of benzoin gave better results than an older one. Since it is probable that this is due to oxidation of the benzoin, a reducing agent, sodium dithionite, $Na_2S_2O_4$, was added to the reaction mixture. With this present the age of the benzoin solution is immaterial. Other reducing agents were tried, but the dithionite gave the best results.

The fluorescence with zinc appears in an alkaline solution in the presence of magnesium hydroxide. This latter material was used in a series of experiments to see if it would absorb fluorescent compounds, as it does titan yellow in the common test for magnesium ion. In this particular case it was highly successful. Subsequent tests showed that the zinc-benzoin fluorescence on the magnesium hydroxide was much more lasting when a small quantity of silicate was present. It is probable that the adsorbing agent is a basic magnesium silicate.

Procedure for the Test

The test solution containing 0.1 mg. or more of zinc ion is made neutral or slightly alkaline with sodium hydroxide. If one is proceeding through the usual scheme of analysis and zinc is precipitated as the sulfide, this is dissolved in hydrochloric acid and boiled until all the hydrogen sulfide is removed. The solution is then made just sufficiently alkaline with sodium hydroxide to dissolve the zinc hydroxide. Any hydroxides insoluble in sodium hydroxide are removed by filtration. The volume of solution should be between 5 and 15 ml. Approximately 1 ml. of each of the following reagents is added in successive order. Medicine droppers delivering about 1 ml. are sufficiently accurate for the measurement.

1. Sodium hydroxide–sodium silicate solution, prepared by mixing 100 ml. of 2.5 *N* sodium hydroxide with 0.6 ml. of 35 per

cent sodium silicate. The sodium silicate is unnecessary if immediate observations are to be made.

2. Sodium dithionite solution, made by dissolving 1 gram of sodium dithionite in 25 ml. of water. This solution must be prepared fresh daily and the quantity is so approximate that the "1 gram" may be measured in a porcelain spoon.

3. Benzoin solution, prepared by dissolving 0.3 gram of benzoin in hot 95 per cent ethyl alcohol.

4. Magnesium nitrate solution, prepared so that 1 liter of solution contains 2 grams of magnesium ion.

After addition of these reagents the solution is shaken and after a minute or two observed under an ultraviolet lamp. A green fluorescence between 4650 and 5700 Å. is a positive test. The G. E. H-4 lamp with a blue-purple bulb is most satisfactory as a source of ultraviolet radiations. If there is much visible light present, it is helpful to make the observation through a piece of green cellophane. For concentrations of zinc less than 0.1 mg. a blank should be run at the same time, because the contrast makes the test more pronounced. The fluorescence increases for the first few minutes after the solutions are mixed and then fades very slowly until it entirely disappears in several hours.

Interferences

Since the zinc test is carried out in a sodium hydroxide solution and all insoluble hydroxides are removed by filtration, it is only the amphoteric cations and certain anions that are likely to cause interference. Beryllium and boron both give a fluorescence with benzoin. The beryllium color range is in the yellow green from 4930 to 5700 Å. and the boron from 4710 to 5700 Å. Both fluoresce before the magnesium hydroxide is added and if the solution is examined under the lamp at this point they will not be confused with zinc. After the magnesium hydroxide is added, zinc can be distinguished from beryllium by comparing to a standard. The boron fluorescence is not apparent in concentrations under one part in 100,000. The only other element causing any difficulty is antimony, which gives a purplish fluorescence that will completely mask the green of the zinc. Since boron and beryllium are seldom found in general qualitative analysis mixtures, and antimony is removed in a previous group, these interferences are not considered serious.

Of the other cations left in solution after a sodium hydroxide treatment only the platinum metals, mercury, silver, and gold, cause difficulty. The ions of these elements are reduced to the metal by the dithionite and a black precipitate results. Consequently they must be removed before the zinc test is applied. Anions causing an interference on being reduced by the dithionite are those of molybdenum, vanadium, tellurium, and selenium. The colored anions such as chromate, permanganate, and the iron cyanides mask the color of the zinc fluorescence and cannot be tolerated. Colorless anions and anions not reduced to precipitates or colored forms by dithionite do not interfere.

All the usual cations and anions were tested as individuals except radium, hafnium, rhenium, and the rare earth group where lanthanum, cerium, praseodymium, and neodymium were the only ones available; the remainder were tested as a group using monazite sand extracts. Some anions, while not causing a fluorescence when alone or with other elements, seem to intensify that of the zinc. In descending order of effectiveness these are: silicate, phosphate, carbonate, and arsenate. Since this order is in fair agreement with the list of adsorbing agents used in chromatographic analysis as given by Strain (*4*), it is believed that the increase in fluorescence is due to the character of the adsorbing agent.

Unknowns containing various mixtures were run following the usual qualitative procedures, and zinc was determined by the fluorescent test outlined above with perfect results in all cases.

Sensitivity of the Test

This test is sensitive to 10 micrograms of zinc ion or to a concentration of one part in a million. This is not so sensitive as dithizone, which Feigl (*1*) claims is good to 0.1 microgram. It is, however, a very pronounced test with a high specificity where results are easily identified.

Discussion

It has not been possible to isolate the fluorescing material for analysis. Unsuccessful attempts were made to remove this from the magnesium hydroxide by means of organic solvents. Nor was it possible to get an indication of the composition of the substance by analyzing for the zinc and benzoin removed from the solution. Tests showed that the zinc was adsorbed from the solution even though benzoin was absent. The quantities involved indicated that $8 \times 10^{-}$ mole of magnesium adsorbed 8×10^{-6} mole of zinc from the alkaline solution. If a greater quantity of zinc was used it was not completely removed. Much of the adsorbed zinc can be easily washed from the magnesium with 2.5 *N* sodium hydroxide; however, some remains after several washings. Benzoin is not adsorbed by the magnesium hydroxide, and even with the zinc present it seems impossible to have all the benzoin retained. Under the conditions of the experiment it required only 1.4×10^{-7} mole of benzoin to produce a fluorescence with 7×10^{-7} mole of zinc, and yet after treating with magnesium and filtering, some of the benzoin but none of the zinc passed into the filtrate. The benzoin was tested for by its fluorescence with boron; this property will be described in detail in a later paper. A lesser quantity of benzoin does not produce a fluorescence but can still be detected in the filtrate after the magnesium hydroxide treatment.

No conclusions as to the formula of the zinc-benzoin complex could be obtained. From the formula of benzoin, it would be expected that the zinc replaces the hydroxyl hydrogen and coordinates with the ketonic oxygen, forming a complex of the type $[Zn(C_6H_5CO.CO.C_6H_5)]^+$. In the filtrations mentioned above the zinc-benzoin complex remains on the filter paper as a highly fluorescent material which loses its fluorescence on drying.

Other adsorbing agents were tried in place of the magnesium hydroxide, but none were successful. Among these were spot test paper, activated alumina, and the hydroxides of cadmium, thorium, and zirconium.

The intensity of the fluorescence varies with the concentration of zinc; however, the differences are not sufficient for quantitative measurement. By comparison to a standard concentrations differing by 10 micrograms at lower concentrations can be distinguished.

Summary

Benzoin in the presence of alkali and magnesium ion reacts with zinc to produce a green fluorescent compound having a spectral range from 4650 to 5700 Å. The reagent is highly specific for zinc; interference is given only by beryllium, boron, and antimony. While the test is not as sensitive as the one using dithizone, it is pronounced and easy to observe.

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Semimicrodetermination of Phosphorus in Organic Compounds

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THE analytical procedure presented below for the determination of phosphorus in organic compounds makes use of the Parr sodium peroxide bomb described previously (*4*). The phosphate is determined volumetrically, the alkalimetric procedure used in steel analysis (*2, 3, 6*) being found applicable with little modification. The precipitate of ammonium phosphomolybdate is collected in a cylindrical filter funnel with fritted-glass disk. The decinormal carbonate-free alkali is standardized empirically against pure potassium dihydrogen phosphate in order so far as possible to exclude uncertainty caused by deviation of the composition of the precipitate from the theoretical. The titration is completed without boiling (*3*).

Reagents

Molybdate Solution. The familiar acid solution (*1, 6*) is used: 118 grams of ammonium molybdate [$(NH_4)_4Mo_7O_{24}.4H_2O$] are dissolved in 400 ml. of water, 80 ml. of concentrated ammonium hydroxide are added, and the solution is poured into a mixture of 400 ml. of concentrated nitric acid and 600 ml. of water, with agitation by passing a current of air through the solution. After several hours the solution is filtered. If thereafter a precipitate of molybdic acid appears the reagent must be filtered before use.

Decinormal Sodium Hydroxide, carbonate-free, standardized against pure potassium dihydrogen phosphate by the procedure described for the analysis. It is convenient to prepare a standard solution of potassium dihydrogen phosphate—e. g., 8.5 grams per liter (0.135 *N*), and to add a 10-ml. aliquot to a solution of 9.0 grams of sodium nitrate, 0.2 gram of potassium nitrate and 1 to 2 ml. of concentrated nitric acid in 50 cc. of water, completing the standardization as described in the procedure starting with the third paragraph.

Decinormal Hydrochloric Acid, standardized against the alkali solution by titration with the latter, using phenolphthalein indicator.

Procedure

If the substance to be analyzed is a solid, transfer to the bomb cup an accurately weighed sample of such size (usually 10 to 30 mg.) that the phosphorus present will lead to consumption of 10 to 20 ml. of decinormal alkali. Add powdered sucrose in such amount that the combined weight of sample and sucrose is 0.2 gram. Introduce 0.2 gram of powdered potassium nitrate and 4.0 grams of sodium peroxide. If the substance to be analyzed is a nonvolatile liquid (the only kind tested), introduce first into the cup the sucrose, potassium nitrate, and sodium peroxide, and then add the sample from a Lunge-Rey pipet (the delivery tip of which is drawn out to a fine opening so as to obtain small drops), determining the weight of sample by difference.

Lock the bomb cover in place, mix the charge, ignite, and extract the cooled melt in water, using the manipulations described in procedures for determination of sulfur (*4*) and of the halogens (*5*). Acidify the alkaline extract by addition of 10 ml. of concentrated nitric acid. The volume at this point should be about 60 ml. The liquid should be clear or nearly so. A small amount of dark carbonaceous material may be without significance, but if the amount is large this may indicate that the decomposition was incomplete, and it may later obstruct the filtration or reduce some ammonium phosphomolybdate.

With the liquid at a temperature of about 40° C. (*3*) (ordinarily the neutralization will warm the liquid to about this temperature) introduce from a pipet 50 ml. of the molybdate reagent. Allow the solution to stand for 2 hours or overnight (*1*).

Using light suction (to minimize danger of loss of ammonia by volatilization) filter the mixture through a filter funnel with sintered-glass disk (a funnel of 15-ml. capacity and 22-mm. inside diameter, and with a disk of "medium" porosity, is suitable), mounted in a Witt suction filtration apparatus. Clear the beaker of the last traces of precipitate by rinsing with 1 per cent potassium nitrate solution, and scrubbing thoroughly. Wash the precipitate on the filter with 10-ml. portions of 1 per cent potassium nitrate solution, applied as a fine stream from a wash bottle. The total volume of the wash solution used in these operations should be 75 ml. Rinse the under side of the filter with water to remove any adhering traces of acid.

Place under the filter funnel (in the Witt apparatus) a 250-ml. Erlenmeyer flask containing 5 drops of 1 per cent phenolphthalein indicator. With suction interrupted measure decinormal sodium hydroxide into the funnel from a 25-ml. or a 50-ml. buret, using enough to dissolve the precipitate, an operation which may be assisted by manipulation with a glass rod. Draw the solution into the flask with suction; if the liquid remains colorless measure from the buret and through the funnel enough additional decinormal alkali to produce a red color. Wash the filter with water. Titrate with decinormal acid to discharge the color, and add a slight excess of acid. Finally titrate with decinormal alkali to a "permanent" pale pink color.

Table I. Determination of Phosphorus in Organic Compounds

Compound	Sample	Sodium Hydroxide	Normality[a]	Phosphorus Found[b]	Phosphorus Calculated
	Mg.	*Ml.*		%	%
n-Butyl phosphate, b. p. 161–163° at 16 mm.	41.61	32.86	0.1091	11.63	11.65
	41.05	32.90		11.80	
	38.15	29.71		11.47	
				Av. 11.6	
Tri - *p* - tolyl phosphate, m. p. 75.1°, corrected	40.75	27.79	0.0913	8.39	8.42
	30.44	20.73		8.38	
	31.33	21.32		8.37	
	16.59	7.95	0.1279	8.26	
				Av. 8.37	
Sodium glycerophosphate, Eastman Kodak Co. No. 644[c]	6.57	4.06	0.1259	10.55	10.37[d]
	11.17	6.79		10.40	10.42[d]
					10.39[d]
					10.45[e]
					10.46[e]
				Av. 10.5	Av. 10.42
Triphenyl phosphite, b. p. 240° at 21 mm.	8.37	4.83	0.1279	9.95	10.00
	9.83	5.66		9.93	
	16.62	9.77		10.13	
				Av. 10.0	
Tri - *m* - tolyl phosphite, b. p. 249–251° at 11 mm.	15.93	8.29	0.1279	8.97	8.81
	18.29	9.13	0.1289	8.67	
	27.24	13.54		8.65	
	22.42	11.33		8.78	
				Av. 8.78	
Tri - *p* - tolyl phosphite, b. p. 260–261° at 10–11 mm.	30.56	15.37	0.1279	8.67	8.81
	9.11	4.66		8.81	
	13.07	6.74		8.89	
	10.78	5.63		9.00	
				Av. 8.83	
Triphenylphosphine, m. p. 80.6° corrected	15.19	10.26	0.1279	11.64	11.83
	14.97	10.16		11.70	
	16.96	11.80		11.99	
				Av. 11.8	

[a] Normality in terms of phosphorus as required by the ratio P = 23 NaOH.

[b] These values are given as calculated, but obviously are valid to not more than three significant figures.

[c] Eastman Kodak Co. designation is "sodium glycerophosphate.$5H_2O$ (52% alpha)". The results obtained indicate a composition midway between the pentahydrate (10.13% P) and the tetrahydrate (10.76% P).

[d] Results by umpire method: sample decomposed by nitric and sulfuric acids, and phosphoric acid determined as $Mg_2P_2O_7$ following double precipitation with magnesia mixture. In test analyses of pure KH_2PO_4 this procedure gave 22.78% P; calcd. is 22.79%.

[e] Results by umpire method: decomposition as above, and phosphoric acid determined as $Mg_2P_2O_7$ following precipitation as ammonium phosphomolybdate and then double precipitation with magnesia mixture. In test analyses of KH_2PO_4 this procedure gave 22.83% P; calcd. is 22.79%.

Results obtained by this procedure applied to seven organic compounds are given in Table I.

The compounds analyzed were all specimens obtained from the Eastman Kodak Company. Sodium glycerophosphate and tri-*p*-tolyl phosphate were analyzed as received. Triphenyl phosphine was twice crystallized from ethanol. The phosphorous esters were distilled under reduced pressure. They all contained some of the corresponding phenols (odor; fore-run during distillation), and when distilled in vacuo they underwent considerable change in the flask, an orange-colored turbidity appearing in the liquid in each case. As small amounts of these materials passed into the distillates, it was necessary to repeat the distillations. In all cases the specimens finally obtained were perfectly clear, and boiled within narrow temperature ranges, but the residual liquids were opaque and much discolored.

Samples were weighed on a semimicrobalance.

Acknowledgment

Appreciative acknowledgment is made to the Faculty Research Committee of the University of Pennsylvania for a grant to aid this study, and to R. R. Hetrick who, under the supervision of W. M. McNabb, executed the accurate umpire analyses of sodium glycerophosphate.

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Determination of Total Sulfur in Rubber

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A new volumetric method has been developed for the determination of sulfate sulfur. The sulfate is reduced to sulfide by treatment with hydriodic acid and the hydrogen sulfide is distilled off and titrated iodometrically. The new method has been applied to the determination of total sulfur in natural and synthetic rubber.

WITH the recent increase in research on natural and synthetic rubber there has arisen a need for a rapid method for the determination of total sulfur in rubber samples where only a semimicrosample can be spared for analysis.

The usual methods in which the rubber is oxidized by digestion with acid, followed in some cases by fusion to complete the oxidation, and the sulfur is precipitated and weighed as barium sulfate, yield good results on semimicrosamples but are time-consuming because quantitative precipitation of the sulfate cannot be attained unless the solution is allowed to stand overnight before filtration. Several volumetric methods, most of which depend upon the precipitation of the sulfur as barium sulfate, have been described, but none is very satisfactory in the determination of small amounts of sulfur.

St. Lorant (*2*) has proposed a volumetric method for the determination of microsamples of sulfate, in which the sulfate is reduced to sulfide with hydriodic acid, and the hydrogen sulfide is distilled off and determined colorimetrically. The author has recently developed a modification of this method which has proved to be very satisfactory in the analysis for sulfur in small samples of rubber. The procedure consists essentially of oxidation of the rubber in the usual manner, reduction of the sulfate to sulfide, separation of the hydrogen sulfide by distillation, and titration of the latter iodometrically.

The new method is very rapid (20 to 25 minutes for the distillation and titration) and is not subject to the errors of adsorption, occlusion, and solubility encountered in the gravimetric barium sulfate method, or to difficulties in detection of the end point encountered in some of the volumetric methods. It possesses the distinct advantage over some of the gravimetric methods, that it is applicable to the analysis of rubber containing barium, lead, and calcium. The only serious objections to the method from the standpoint of general use are that hydriodic acid is expensive and that the applicability of the method is somewhat limited because of the fact that no more than about 5 mg. of sulfur can be handled conveniently. This means that the sample size must be small when analyzing rubber high in sulfur; and when small samples are used there is always the danger of error due to unequal distribution of sulfur in the sample.

Apparatus

The apparatus is shown in Figure 1. It consists of a 125-ml. Erlenmeyer flask with standard taper 24/40 joint, a distillation head with capillary pressure regulator, and a 300-ml. Erlenmeyer flask with standard taper 24/40 joint. The capillary tubing is 2 mm. in inside diameter and extends to within 2 to 4 mm. from the bottom of the 125-ml. flask.

Reagents

Nitric Acid–Zinc Oxide–Bromine Mixture. Dissolve 10 grams of zinc oxide in 100 ml. of nitric acid and saturate with bromine.

Acid Mixture for Distillation. Place 160 ml. of hydriodic acid (specific gravity 1.70), 160 ml. of hydrochloric acid, and 45 ml. of hypophosphorous acid (50 per cent) in a 500-ml. Erlenmeyer flask. (If the hydriodic acid contains hypophosphorous acid as a preservative use only 40 ml. of hypophosphorous acid.) Add a few grains of silicon carbide and boil vigorously without cover for 5 minutes. Cool in an ice bath to room temperature. Keep stoppered in a 500-ml. brown glass-stoppered bottle to avoid oxidation of the hydriodic acid.

Ammoniacal Cadmium Chloride Solution. Dissolve 10 grams of cadmium chloride dihydrate in water. Add 500 ml. of ammonium hydroxide and dilute to 5 liters.

Standard Potassium Iodate Solution (0.01 *N*). Recrystallize c. p. potassium iodate from water twice and dry at 180° C. to constant weight. Weigh 0.7134 gram of the pure potassium iodate and dissolve in water. Add 2 grams of sodium hydroxide and then 10 grams of potassium iodide (free from potassium

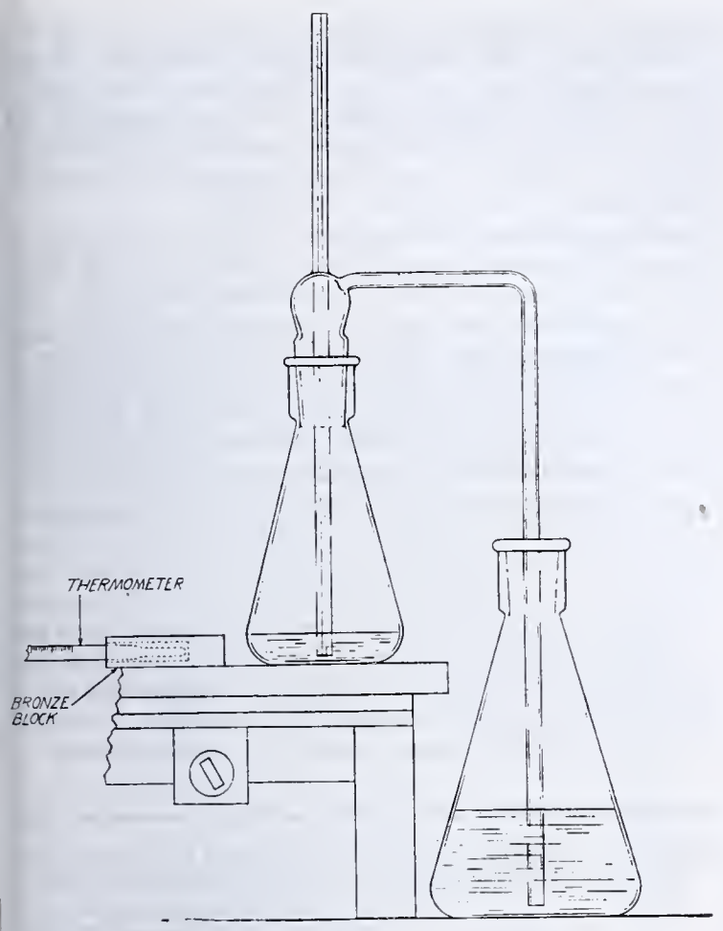

FIGURE 1

iodate). After complete solution of all salts adjust to room temperature and dilute to 2 liters in a volumetric flask.

STANDARD SODIUM THIOSULFATE SOLUTION (0.01 *N*). Dissolve about 5 grams of sodium thiosulfate pentahydrate in 2 liters of freshly boiled and cooled distilled water. Store in a clean Pyrex bottle. To standardize this solution, pipet 25 ml. of the standard potassium iodate solution into a 300-ml. Erlenmeyer flask. Add 200 ml. of water and 20 ml. of hydrochloric acid. Allow to stand 1 minute and then titrate with the thiosulfate solution. As the end point is approached add 10 ml. of 0.2 per cent starch solution and titrate carefully until the solution is colorless. The thiosulfate solution should be restandardized every 15 days.

STARCH SOLUTION. Add a cold aqueous suspension of 2 grams of soluble starch to 1 liter of boiling water. Cool to room temperature and store in a clean bottle.

Procedure

Place 0.050 to 0.100 gram of the rubber sample, which has been either "crumbed" or cut into as small pieces as possible, in a 125-ml. Erlenmeyer flask with ground-glass joint. (The method as written is applicable to the analysis of semimicrosamples of rubber. If an analysis of larger samples is desired appropriate increases in the amounts of reagents must be made. It is best, however, to limit the amount of sulfur in the sample to 5 mg. or less.)

(When highly saturated material such as Butyl rubber is to be analyzed, it is desirable to limit the sample size to 0.05 to 0.10 gram in order to decrease the time of oxidation and danger of explosion with perchloric acid. In general, oxidation of difficultly oxidizable material is more rapid and convenient with the perchloric acid method than with the A. S. T. M. method.)

Proceed by either of the following methods:

A. S. T. M. METHOD. Add 4 ml. of nitric acid–zinc oxide–bromine mixture. Cover and heat on a steam bath or low-temperature hot plate to decompose the sample. When rapid solution ceases add 3 ml. of fuming nitric acid. Cover, and, while swirling the flask to prevent ignition of the sample, heat on a hot plate with surface temperature maintained at 180° to 200° C. When danger of ignition is past, allow the solution to boil gently until most of the organic matter is destroyed and the acid is all but expelled. With difficultly oxidizable samples such as Buna S and Butyl rubber it may be necessary to add more fuming nitric acid and take down to near dryness 2 or 3 times before enough of the sample has been oxidized to make it safe to continue. Finally, remove the cover and bake over a Tirrill flame until the zinc nitrate is converted to zinc oxide and most of the brown fumes are expelled. Return the flask to the hot plate to cool and to allow the remaining fumes to be expelled. Cool to 30° C. Wash down the sides of the flask with 5 ml. of hydrochloric acid and boil without cover to expel about half of the acid. It is essential that all nitrates be expelled in order to avoid interference in the titration described below. Cool to room temperature and reserve the solution for the distillation.

PERCHLORIC ACID METHOD. Proceed as directed in the A. S. T. M. method. When most of the readily oxidizable organic matter has been oxidized, and all but 1 or 2 ml. of the acid has been expelled, add 5 ml. perchloric acid (60 per cent). Cover and boil gently at a hot-plate temperature of 180° C. to 200° C. until white perchloric acid fumes appear. During this boiling it is essential that the flask be completely covered with a watch glass. With difficultly oxidizable material such as Butyl rubber considerable amounts of organic material may remain after expulsion of the nitric acid. If this occurs, the oxidation with perchloric acid should be allowed to proceed only to the point where the sample begins to char. At this point add more fuming nitric acid and repeat the evaporation. Repeat if necessary. Finally, when most of the organic matter is destroyed, the sample can safely be heated until fumes of perchloric acid appear without danger of ignition or explosion. Fume vigorously on a flame without cover to expel all but about 2 ml. of the acid. Cool to room temperature and reserve for the distillation.

After oxidation of the sample, add about 5 grains of silicon carbide (12-mesh grains that have been boiled in hydrochloric acid) to prevent bumping, and 35 ml. of the acid mixture and immediately cap with the distillation head. Place on a hot plate with surface temperature of 375° C. to 400° C. and boil for 5 minutes, catching the distillate in 150 ml. of ammoniacal cadmium chloride solution in a 300-ml. Erlenmeyer flask with ground-glass joint. The boiling should be timed from the moment fumes of ammonium chloride are first seen in the 300-ml. flask.

Remove the small flask from the plate and detach the distillation head. (If difficulty is encountered in removing the head, cool the joint and then run hot water on it.) Cool the distillate rapidly in an ice bath to 10° C. (In the analysis of samples containing over about 5 mg. of sulfur, add an excess of potassium iodate to a 125-ml. flask containing 20 ml. of hydrochloric acid and 20 ml. of water. Pour this solution in one stroke into the flask containing the sample. Stopper immediately and shake to oxidize all the sulfide in the flask. Wash the remains of the iodine into the large flask and back-titrate with thiosulfate as directed.)

Add 20 ml. of hydrochloric acid and swirl once to mix thoroughly. Titrate immediately and rapidly with potassium iodate solution, using a rapidly flowing buret—(i. e., a 50-ml. buret which will empty by gravity in 60 to 70 seconds)—until an excess of about 1 or 2 ml. has been added, as indicated by the yellow color. Cap immediately with a ground-glass stopper and shake vigorously to entrap any hydrogen sulfide in the atmosphere in the flask. Allow the potassium iodate buret to stand 1 minute after the titration before reading it. Add 10 ml. of starch solution and titrate with standard sodium thiosulfate solution (0.01 *N*). Carry a blank through the whole procedure. The blank should not be greater than 0.1 ml. of potassium iodate solution (0.01 *N*):

$$\frac{[(\text{Ml. of } KIO_3 - \text{ml. of } Na_2S_2O_3 \times KIO_3 \text{ factor}) - \text{blank}] \times 0.01603}{\text{Sample weight in grams}} = \text{per cent sulfur}$$

Discussion

The proposed volumetric method for the determination of sulfate yields satisfactory results when the amount of sulfur to be determined is less than about 10 mg. although slightly low values are obtained when the amount of sulfur present exceeds about 1 mg. (see Table I). With quantities greater than about 5 mg. special precautions must be taken to avoid loss of hydrogen sulfide during the titration.

Low results are obtained if the hydrogen sulfide is allowed to escape during the distillation or titration, if the temperature of distillation is too low, if the concentration of hydriodic acid is too low, and if nitrates in quantities greater than traces are present at the distillation. High results may be obtained

Table I. Determination of Sulfur in Potassium Sulfate by the Proposed Volumetric Method

No.	Sulfur Present	Sulfur Found	Error
	Mg.	*Mg.*	*Mg.*
1	0.06	0.07	+0.01
2	0.13	0.13	±0.00
3[a]	0.13	0.13	±0.00
4	0.25	0.25	±0.00
5	0.62	0.61	−0.01
6[b]	0.62	0.62	±0.00
7	1.25	1.24	−0.01
8[a]	1.25	1.22	−0.03
9[b]	1.25	1.23	−0.02
10[c]	1.25	1.22	−0.03
11[d]	1.25	1.22	−0.03
12[e]	1.09	1.08	−0.01
13	3.12	3.10	−0.02
14[b]	3.12	3.09	−0.03
15	6.23	6.16	−0.07
16[b]	6.23	6.20	−0.03

[a] Titrated with KIO_3 using CCl_4 as indicator.
[b] No $HClO_4$ present in acid mixture.
[c] 0.2 gram of $BaCl_2.2H_2O$ present.
[d] 0.05 gram of $PbCl_2$ present.
[e] Different K_2SO_4 solution used.

unless a blank correction for the reagents is made. Because of the danger of exceptionally heavy contamination of the hypophosphorous acid with sulfur compounds, it is necessary to resort to a preliminary distillation of the acid mixture in order to reduce the blank to a reasonable figure.

In preliminary work the usual distillation apparatus for the evolution method for sulfur in steel was used. This proved unsatisfactory because the variation in the diameter of the neoprene rubber stoppers and the neck of the Erlenmeyer flasks was so great that the distance between the bottom of the flask and the end of the capillary pressure regulator could not be conveniently controlled. In the apparatus finally chosen the rubber stopper was replaced by a standard taper ground-glass joint.

When hydrogen sulfide is titrated directly with standard iodine using starch as indicator the end point is very poor, owing to the appearance, in the solution, of a permanent reddish color which obscures the blue of the end point. The color is probably caused by the adsorption of some compound of iodine and sulfur on the starch molecule. As evidence of this it is noted that very little free sulfur appears during the titration of those solutions which contain starch. Experiments indicated that the red color is more pronounced the colder the solution and the greater the salt concentration, especially if the salt is an iodide. It was found that in the titration of hydrogen sulfide in solutions containing but small quantities of salts the red color could be almost completely eliminated by adding about 0.5 mg. of mercuric iodide or mercuric chloride to the solution or by titrating at 35° C. to 40° C. Unfortunately, the addition of mercury caused the results to be slightly low, probably because of the conversion of part or all of the mercury to sulfide. Because of this, and the fact that the use of mercury salts is not very effective in the presence of appreciable quantities of salts, the problem was not considered further.

The best method for titrating hydrogen sulfide is that in which an excess of iodine is added, followed by back-titration with thiosulfate using starch as indicator. This method has the advantage that the end point is good and the danger of loss of hydrogen sulfide can be greatly reduced by rapid overtitration with iodine using a fast flowing buret.

It will be seen in the procedure that no extra iodide is added to the solution previous to titration, as is sometimes done in direct titrations of sulfide with iodine. This addition is unnecessary and in fact is purposely omitted in the method because a considerable amount of hydriodic acid comes over with the hydrogen sulfide, and any further addition of iodide is detrimental to the starch-iodine end point.

The two gravimetric methods most often used for the determination of sulfur in rubber stocks which are free from barium and large amounts of lead and calcium are those of Wolesensky (*3*, hereinafter called the perchloric acid method), and Kratz, Flower, and Coolidge (*1*, hereinafter called the A. S. T. M. method). In the analysis of representative samples of natural and synthetic rubber stocks these two methods give results which are in good agreement. In like manner it was found (see Table II) that results obtained by the proposed volumetric method and the perchloric acid method check very well, although as would be expected from the data in Table I, the volumetric method gives somewhat lower results.

In agreement with Wolesensky it was found that great care must be taken to prevent loss of sulfur during the digestion of rubber samples with nitric and perchloric acids. The loss is negligible if the sample is digested slowly in a long-necked Kjeldahl flask. When a small Erlenmeyer flask is used, however, the loss may amount to 1 or 2 per cent (when the new volumetric method is used for the final determination), unless the digestion is slow and the flask is kept completely covered with a watch glass until all organic matter is expelled. In the procedure an attempt has been made to minimize this loss by delaying the addition of the perchloric acid until most of the readily oxidizable material has been oxidized. After oxidation of the organic material the sample can be taken to copious fumes of perchloric acid without loss of sulfur, providing an excess of zinc is present. This suggests that the sulfur, which is lost during the digestion of the organic matter, has not been oxidized to sulfate. No loss of sulfur is encountered when the A. S. T. M. method is used.

Table II. Determination of Total Sulfur in Natural and Synthetic Rubber Using Proposed Volumetric Method

No.	Rubber Sample	Per Cent Sulfur Found: Gravimetric $HClO_4$ method	New Volumetric Method $HClO_4$ oxidation	New Volumetric Method A. S. T. M. oxidation
1	Buna S gum stock	0.19	0.19	...
2		0.19	0.20	...
3	Gum rubber 1	0.63	0.62	0.63
4		0.63	0.62	0.62
5	Gum rubber 2	1.88	1.87	1.85
6		1.88	1.86	1.87
7	Gum rubber 3	3.55	3.52	3.50
8		3.55	3.48	3.50
9		3.55	3.47	3.48

Experimental

A standard solution of potassium sulfate was prepared by dissolving 0.6780 gram of the pure dry salt in water and diluting to 1 liter in a volumetric flask. Aliquot portions of the solution were evaporated to dryness in 125-ml. Erlenmeyer flasks. The samples were then analyzed for sulfur as directed in the procedure, except that an acid mixture of the following proportions was used: 100 ml. of hydriodic acid (specific gravity 1.70), 100 ml. of hydrochloric acid, 25 ml. of hypophosphorous acid (50 per cent), and 25 ml. of perchloric acid (60 per cent). The results are shown in Table I.

Four representative types of rubber stocks (containing no barium, lead, or calcium) were analyzed for sulfur as directed in the procedure. Results were obtained using both the A. S. T. M. and the perchloric acid methods of oxidation. The samples were also analyzed by the gravimetric perchloric acid method. The results are given in Table II.

Acknowledgment

The author wishes to express his gratitude to B. L. Clarke and M. L. Selker of these laboratories, who read the manuscript and offered several valuable suggestions.

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Determination of Combined Formaldehyde in Organic Compounds and in Cellulose Formals

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Combined formaldehyde can be determined in organic compounds and in cellulose formals by a method that omits distillation and determines formaldehyde in the hydrolyzate by Blaedel and Blacet procedure adapted to photoelectric colorimeter.

TANDARD methods for the determination of formalde-) hyde usually require distillation with acid in order to iso- te the formaldehyde. Organic compounds containing mbined formaldehyde yield formaldehyde quantitatively hen hydrolyzed in the presence of sulfuric acid. In the ethod presented distillation is omitted and the formaldehyde determined in the hydrolyzate by the Blaedel and Blacet) procedure adapted to a photoelectric colorimeter in which magenta color is developed by use of Schiff's reagent. Cor- ct results may be obtained even in the presence of consider- ble amounts of other aldehydes. Amounts of formaldehyde etween 0.01 and 0.10 mg. may be determined in 5 ml. of lution. Thus the method is applicable to large samples ntaining low concentrations or to small samples containing gh concentrations of formaldehyde (Table I).

TABLE I. TYPICAL ANALYTICAL DATA

Compound	Formaldehyde Theory %	Formaldehyde Found %	Error %
imethylene-*d*-mannitol, m. p. (corr.) 232° C., $C_9H_{14}O_6$	41.29	41.2	−0.2
		41.2	−0.2
iacetyldimethylene-*d*-mannitol, m. p. (corr.) 164–5° C., $C_{12}H_{18}O_6$	20.69	20.3	+0.5
		20.8	+0.5
imethylene-*d*-dulcitol, m. p. (corr.) 248–9.5° C., $C_8H_{14}O_6$	29.13	29.1	−0.1
		29.0	−0.4
iacetyldimethylene-*d*-dulcitol[a], m. p. (corr.) 256–62° C., $C_{12}H_{18}O_8$	20.69	20.0	−3.3
		20.0	−3.3
imethylene-*d*-sorbitol, m. p. (corr.) 204–6° C., $C_9H_{14}O_6$	41.29	40.9	−0.9
rioxymethylene, $C_3H_6O_3$	100.0	99.6	−0.4
		100.3	+0.3
thylal, $C_5H_{12}O_2$	28.84	28.4	−1.6
ellulose formal (1)	0.35[b]	0.34	−2.9
		0.34	−2.9
ellulose formal (2)	1.96[b]	1.99	+1.5
		1.99	+1.5
-Methylethylidene glucoside, m. p. (corr.) 186–8° C., $C_9H_{16}O_6$	0.0	0.0	0.0
ellulose	0.0	0.0	0.0
ormaldehyde, 14 mg. per liter + 200 mg. of acetaldehyde	100.0	100.3	+0.3
ormaldehyde, 14 mg. per liter + 200 mg. of propionaldehyde	100.0	100.3	+0.3
cetaldehyde (200 mg.)	0.0	0.0	0.0
ropionaldehyde (200 mg.)	0.0	0.0	0.0

[a] Compound probably impure. Haskins, Hann, and Hudson (*2*) give . p. (corr.) of 264–265° C
[b] Values obtained by distillation followed by dimedone precipitation ethod.

With 12 *N* sulfuric acid, a constant maximum yield of ormaldehyde is reached in 16 hours or less from cellulose ormals at room temperature, but 16 days are required to ttain theoretical yields of formaldehyde from trimethylene- -mannitol and similar compounds. For lower concentrations f acid longer time is required. However, on heating at 0° C. these latter compounds, as well as cellulose formals, ield formaldehyde quantitatively in 2 hours (Table II). urther heating of cellulose formals with 12 *N* sulfuric acid produces darkening of the solutions and gives lower values. For this reason, heating for 2 hours at 90° C. is recommended as a general procedure, but for cellulose formals (samples analyzed contained less than 2 per cent of formaldehyde) overnight treatment with 12 *N* sulfuric acid at room temperature is satisfactory.

TABLE II. FORMALDEHYDE YIELD AT 90° C.

Material	Formaldehyde Theory	2 hours	5 hours	8 hours	16 hours	24 hours
Trimethylene-*d*-mannitol	41.29	41.4	40.9	40.8	41.2	41.9
		41.1	40.7		40.7	39.9
Dimethylene-*d*-dulcitol	29.13	29.1	29.0	29.2	28.8	28.6
		29.0	28.9		28.6	28.3
Cellulose formal	1.52	1.53	1.40	1.28	..	..

The size of sample is selected to yield a concentration of formaldehyde of approximately 0.05 mg. in 5 ml. of the hydrolyzate. It is desirable to set up a series of standards containing amounts of formaldehyde ranging from 0.01 to 0.10 mg. in 5 ml. and to develop the color in them at the same time as in the unknowns by adding 6 ml. of 10 *N* sulfuric acid and 10 ml. of the fuchsin-sulfite reagent. This reagent (*1*) is prepared by dissolving 0.5 gram of fuchsin (rosaniline hydrochloride) in 500 ml. of water, adding 5.15 grams of sodium acid sulfite, allowing to stand 15 minutes, adding 17 ml. of 6 *N* hydrochloric acid, and allowing to stand at least 3 hours before using. The reagent is stable for at least a month. The tubes are allowed to stand from 2 to 2.5 hours, during which time the color given by higher straight-chain aldehydes and their polymers fades completely, though fading of the color given by formaldehyde is not appreciable (*1*). The transmission is then determined and plotted against milligrams of formaldehyde to give a curve like the one shown in Figure 1. The formaldehyde solution used for the preparation of the standards is standardized by dimedone (5,5-dimethylcyclohexanedione-1,3) precipitation according to Yoe and Reid (*4*).

When color is developed by adding fuchsin-sulfite reagent to known amounts of formaldehyde, a definite and reproducible curve rather than a straight line is obtained when either the transmission or the logarithm of the transmission is plotted against the concentration. Other color reagents for formaldehyde, such as phloroglucinol in basic solution and phenylhydrazine hydrochloride in either acid or basic solution, also fail to give straight lines and the curves are not so nearly reproducible as those obtained with fuchsin-sulfite reagent.

Experimental

Crystalline formals of known composition were prepared from the corresponding alcohols by the method described by Haskins, Hann, and Hudson (*2*). They were purified to constant melting points by recrystallization and their identities were further verified by carbon and hydrogen determinations. The ethylal was purified by distillation.

The data presented in Table I were obtained by weighing accurately 20 to 40 mg. of the crystalline compounds, transferring to 1-liter volumetric flasks, and dissolving in and making to volume with 12 *N* sulfuric acid. Portions of the solution were poured into glass-stoppered 250-ml. Erlenmeyer flasks, stoppered tightly, and placed in a constant-temperature oven at 90° C. After 2 hours the flasks were removed and cooled, and 5-ml. aliquots were pipetted into matched tubes of an Evelyn photoelectric colorimeter, provided with filter No. 565 (Corning Glass Co.). To each were added 6 ml. of water (6 ml. of 10 *N* sulfuric acid to the standards) and 10 ml. of the fuchsin-sulfite reagent. After addition of the reagent, the tubes were allowed to stand from 2 to 2.5

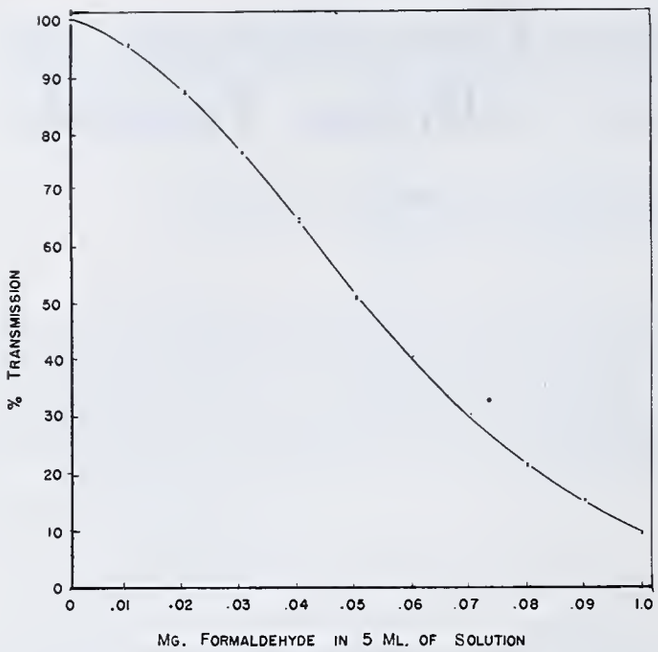

FIGURE 1. TYPICAL WORKING CURVE

hours and the transmission was determined. Spectrophotometric curves of the color developed indicated that filter 565, whose transmission limits are 550 to 585 mμ, is the correct filter to use.

In the case of cellulose formals, about 0.1 gram of the material was accurately weighed and placed in 250-ml. glass-stoppered Erlenmeyer flasks. Exactly 100 ml. of 12 *N* sulfuric acid were added to each flask, and the flasks were placed in a constant-temperature oven at 90° C. for 2 hours or allowed to stand overnight at room temperature. Formaldehyde was determined on 5-ml. aliquots of the solution as described above. Solution of the sample was not necessary for correct results. Since cellulose formals of known composition were not available, the results obtained by the method described here were compared with the values determined by distilling the formaldehyde by the method of Wood (*3*), followed by determination of formaldehyde in an aliquot of the distillate by dimedone precipitation according t Yoe and Reid (*4*).

The data in Table I show that acetaldehyde or propionald hyde did not interfere with the determination. β-Methy ethylidene glucoside and cellulose, both of which produc aldehyde groups but no formaldehyde on acid hydrolysis, gav zero values. Good agreement was obtained between th analysis of cellulose formals by the colorimetric and dimedon gravimetric methods.

In order to determine how small a sample it was practical t use, 5.840- and 6.207-mg. samples of trimethylene-*d*-mannit were weighed on the microbalance, placed in 250-ml. volumetri flasks, and made to volume with 12 *N* sulfuric acid. The flask were placed in a constant-temperature oven at 90° C. for 2 hou and cooled. Using 5-ml. aliquots and the above-describe colorimetric procedure, values of 40.6 and 41.0 per cent formalde hyde were obtained. Samples of 0.062 and 0.127 mg., smalle than those commonly used for microanalysis, were weighed o the microbalance, placed in matched tubes of the colorimeter, an 5-ml. portions of 12 *N* sulfuric acid were added with a pipet. Th stoppered tubes were heated at 90° C. for 2 hours and the colo was developed, giving values of 39 and 40 per cent of formalde hyde, respectively. Considering the very small samples usec these values are in satisfactory agreement with the theoretic value of 41.29 per cent.

Acknowledgments

The authors are indebted to the following members of thi laboratory: James H. Kettering, Lamont Hagan, and J David Reid for the cellulose formals; Richard E. Reeves fo the β-methylethylidene glucoside; and Robert T. O'Conno for the spectrophotometric curve of the color developed.

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NOTE ON ANALYTICAL PROCEDURE

Stability of the Nickelous-Ammonia Color System

J. P. MEHLIG, Oregon State College, Corvallis, Ore., AND R. E. KITSON, Purdue University, Lafayette, Ind.

IN DEVELOPING a colorimetric method for the determination of nickel in steel with ammonia Ayres and Smith (*1*) found no change in the color of the system after 150 hours. In a spectrophotometric study of this method the senior author of this paper found that there was no evidence whatever of fading or other color change over a period of 4 weeks (*2*).

For the purpose of further study the six solutions which had been used in this test (*2*), containing 100, 200, 300, 500, 1000, and 1500 p. p. m. of nickel in 1.5 *M* ammonium hydroxide, were allowed to stand 55 weeks longer in glass-stoppered Pyrex bottles in diffuse light. Spectral transmission curves were then made with the self-recording photoelectric spectrophotometer at Purdue University, and, when compared with the curves given by the corresponding freshly prepared solutions, were found to be very similar. From the transmittancy at 582 mμ, the wave length of maximum absorption for this system, the percentage error in the apparent concentration of nickel was calculated (*2*) by use of the special color slide rule.

The range of apparent change in concentration of the nickel was from −0.7 to +7.1 per cent (average 3.0 per cent), thus showing that the color of the system is practically unchanged after 59 weeks. The percentage error is less in the more concentrated solutions (*3*). Such marked stability makes possible the use of a series of permanent standards, which must, however, be kept tightly stoppered to prevent loss of ammonia. The action of ammonia on the glass is reduced to a minimum when Pyrex containers are used.

Acknowledgment

The writers wish to thank M. G. Mellon of Purdue University, in whose laboratory this work was done, for the privilege of using the Purdue spectrophotometer.

Literature Cited

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(2) Mehlig, J. P., *Ibid.*, 14, 289 (1942).
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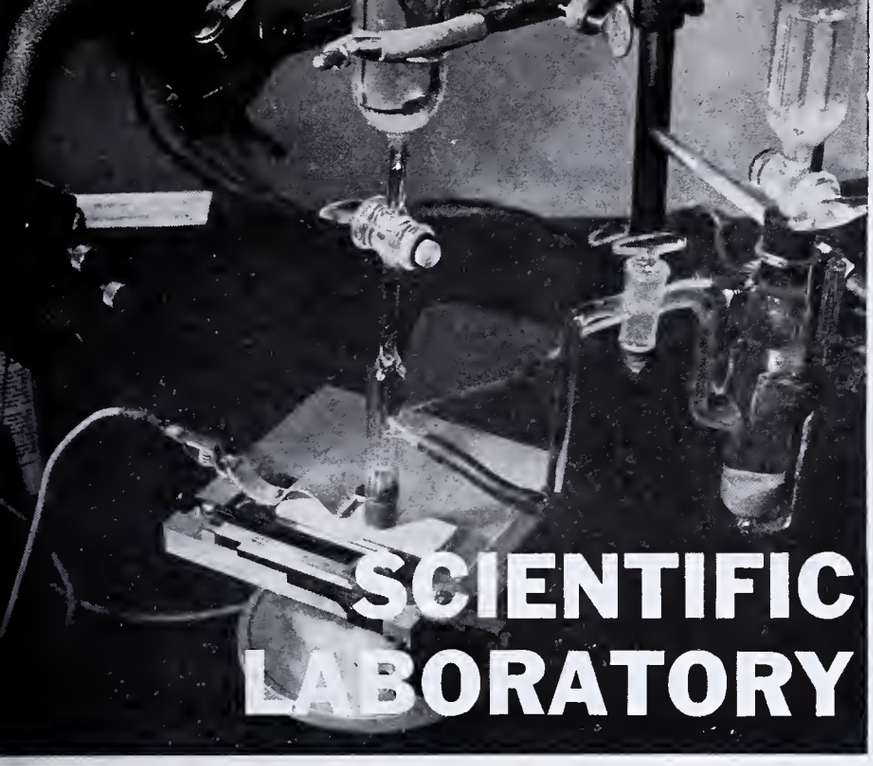
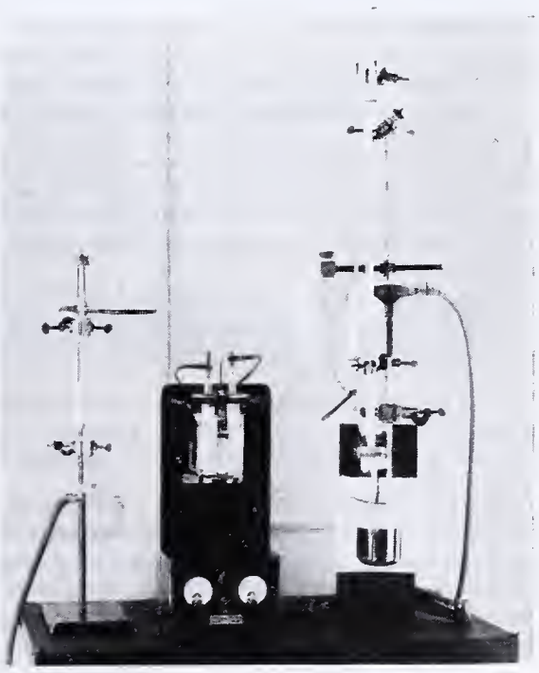


SCIENTIFIC APPARATUS LABORATORY EQUIPMENT

Electro-Stripper

The efforts to reduce the thickness of tin coating applied to steel eet have necessitated new methods of analysis in the laboratory, errors inherent in older methods are proportionately higher when e thickness of the tin coating is reduced. The Bendix Electro-Stripper has been developed for the determination of tin coating according to the method developed by Bendix, Stammer, and Carle [Ind. Eng. Chem., Anal. Ed., **15**, 501 (1943)] and is available from the Wilkens-Anderson Co., 111 North Canal St., Chicago 6, Ill. With it one operator may handle 200 determinations in one day.

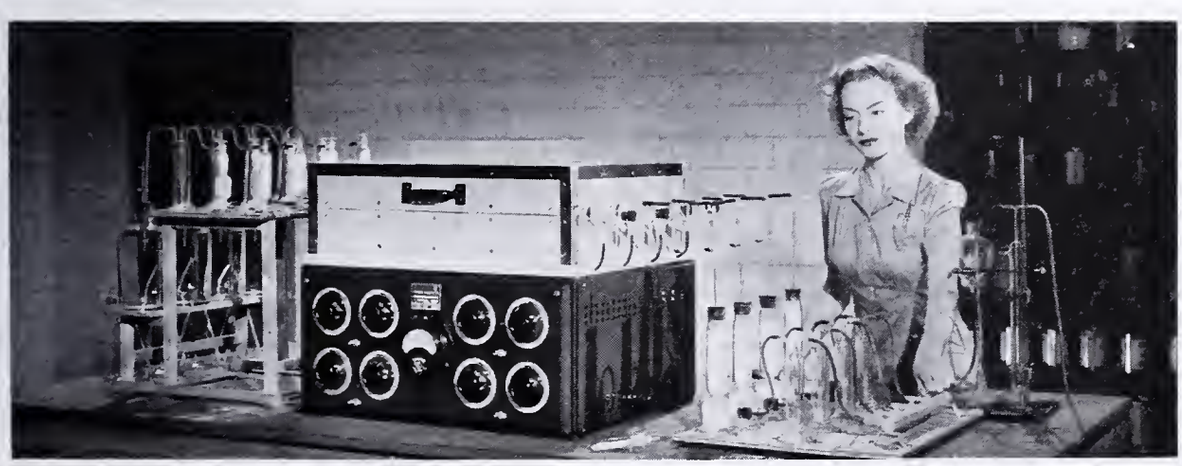


Multiple-Unit Combustion Furnace

Incident to the stepped-up production of high-octane aviation soline essential to the war program, the Universal Oil Products ., Chicago, Ill., has developed a multiple-unit electrically heated mbustion furnace offering several novel features. The equipment manufactured by the Precision Scientific Co., 1750 North Spring-ld Ave., Chicago.

Although designed specifically for the determination of carbon on cking catalysts used for producing high-octane aviation gasoline, e equipment is modifiable to handle a wide variety of organic com-stions within the temperature limit of 540° C. (1000° F.).

Briefly, the equipment comprises (1) oxygen-purification system for mbustion gases; (2) oxygen pressure-regulating column; (3) ctrically heated furnace 24 inches long, with four combustion tubes nch in diameter and 30 inches long. Heating intensity for each half each tube is independently controlled, and temperature of any tube n be readily observed by means of an indicating pyrometer; (4) nventional absorption trains with calcium chloride for absorption moisture and Ascarite for removal of carbon dioxide.



BOOK REVIEWS

Frontiers in Chemistry, Volume I. The Chemistry of Large Molecules. Edited by *R. E. Burk* and *Oliver Grummitt.* xii + 313 pages. Interscience Publishers, Inc., New York, N. Y., 1943. Price, $3.50.

This is a collection of the substances of 12 lectures, presented by six outstanding American chemists at Western Reserve University early in 1942.

In the first chapter, by H. Mark, the mechanism of polymerization reactions is ably discussed, with emphasis on the kinetic theory equations on which the interpretation of such reaction mechanisms is based and on the assumptions underlying them. In the second chapter, Professor Mark presents a clear and useful summary, in nontechnical language, of the results of x-ray investigations of high polymers.

E. O. Kraemer contributes an interesting discussion of the viscosity of solutions of macromolecules and of the diffusion of these molecules in such solutions. He then presents an authoritative critical account of the application of the ultracentrifuge to the study of the sizes and shapes of large molecules.

A. Tobolsky, R. E. Powell, and H. Eyring, in an outstanding chapter on "Elastic-Viscous Properties of Matter", first outline, with the appropriate mathematical equations, a general procedure for the semiquantitative theoretical treatment of properties involving flow; they then apply this procedure, and others involving statistical methods, to the interpretation of various properties of systems containing giant molecules. Rubberlike elasticity and the viscosity and thermodynamic properties of solutions of long-chain polymers are especially considered.

R. M. Fuoss presents an excellent treatment of "The Electrical Properties of High Polymers", showing how the variation of dielectric constant and loss factor with temperature and with frequency is related to the polymer structure.

C. S. Marvel gives a fine discussion of the "Organic Chemistry of Vinyl Polymers", dealing especially with the evidence, obtained by classical organochemical methods, regarding the sequence of groups in vinyl polymer chains.

E. Ott, in the final chapter, contributes an admirable summary of the "Chemistry of Cellulose and Cellulose Derivatives", correlating the chemical properties of these substances with their structures.

Western Reserve University, the publishers, the editors, and the authors are all to be congratulated on this interesting and useful addition to the literature on giant molecules.

MAURICE L. HUGGINS

Inorganic Qualitative Analysis. *H. A. Fales* and *Frederic Kenny.* First edition. ix + 237 pp., 39 figures, 14 × 21 cm. D. Appleton-Century Co., New York, N. Y., 1943. Price, $2.65.

The announced purpose of this book "is to present some important fundamental principles of chemistry and to use as a framework for the study of these principles well-tried and well-established qualitative analytical procedures".

The principles, covering seven chapters and including a total of 155 numerical problems, deal with general considerations, types of compounds, strong and weak electrolytes, hydrolysis of salts, solubility product, complex ions, and oxidation-reduction theory. This presentation seems adequate for a professed one-semester book. A schedule is included to correlate the class and laboratory work.

Laboratory directions, for work on a semimicro scale, cover 74 pages. Conventional procedures include 23 cations, but, as usual, the alloying metals in steel are slighted. The anions are limited to four—chloride, nitrate, sulfate, and carbonate. Obviously, re qualitative analysis of complicated unknowns is not contemplate Each group of procedures includes supplementary experiments d signed to illustrate the fundamental principles emphasized.

As a whole, the book reflects the careful writing characterizing t authors' previous text on quantitative analysis. It is clear, concis and conservative. To the reviewer, it looks teachable in the kin of course for which it was written.

M. G. MELLO

Methods for Tungsten Analysis

The Bureau of Mines has published a report on methods analyzing tungsten ores, particularly those from low-grade d posits now being explored because of a great wartime deman for the steel-hardening metal.

Analyses of the tungsten content of low-grade ores ofte have varied widely and have led to confusion. Dependable metl ods of analysis applicable to all types of these ores are essentia to determine which are worth treating and to regulate th method of treatment to assure the best recovery. To fill th need the report describes in detail three methods tried an proved in the Bureau of Mines laboratory at Salt Lake City the standard cinchonine method, the tannic acid—antipyrin method, and a third method combining the first two.

The combination method was found most suitable for all typ of low-grade tungsten ores and now is in use at the laborator The time required for analysis is less, the amount of cinchonin needed is reduced, and the final precipitation of tungsten i variably is complete.

A procedure for recovering the cinchonine, a reagent n readily available, also is outlined.

A copy of Report of Investigation 3709, "The Determinatio of Tungsten in Low Grade Ores", by H. E. Peterson and W. I Anderson, may be obtained from the Bureau of Mines, Depar ment of the Interior, Washington, D. C.

WPB Form for Reagent Chemicals

A standard form of certification as to the uses of reager chemicals ordered by research and other laboratories is provide by Order P-135-A, issued August 5 by the War Production Boar The order also provides that each laboratory shall be entitled the full small-order exemption.

Under the standard form the purchaser certifies to the sell and to WPB "that the reagent chemicals called for by this ord will be used, or resold for use, in a laboratory for one or more the following purposes: analysis, testing, control, educational, research".

NDUSTRIAL AND ENGINEERING CHEMISTRY

ANALYTICAL EDITION

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Analysis by Infrared Spectroscopy

A New Method Applied to Mixtures of Nitroparaffins

J. RUD NIELSEN AND DON C. SMITH[1]
University of Oklahoma Research Institute, Norman, Okla.

A method of analysis by infrared spectroscopy is described, which is applicable, if Beer's law holds, and a set of wave lengths can be found such that at each wave length only one component of the mixture has strong absorption while the other components have weak absorption. This method gives a high degree of accuracy with a minimum of computational work. Its application to certain ternary mixtures of the four lowest nitroparaffins is described.

RECENT improvements in apparatus and technique have made it feasible to apply infrared absorption spectra to the nalysis of mixtures of chemical compounds (*1*, *2*, *3*, *9*). In everal industrial laboratories isomeric and other mixtures of ompounds which are difficult to differentiate by other means are ow analyzed in this manner.

Infrared methods of quantitative analysis of binary mixtures re particularly simple and can be applied even when the mixures do not follow Beer's law. Wright (*9*) has discussed several rocedures suitable for industrial use.

When more components are present it may be possible to find r each compound a wave length at which that compound has rong absorption while all the other compounds have negligible osorption. When that is the case each component can be dermined separately by the simple methods used for binary mixres (*1*). Unfortunately, this condition can seldom be realized ith sufficient accuracy, and the general methods of analysis of ulticomponent mixtures involve much time-consuming computional labor.

In many cases, however, a set of wave lengths can be found ich that at each wave length only one component of the mixture as strong absorption while the other components have weak bsorption. In the present paper a simple and practical method f analysis is described which is applicable to such cases, proided Beer's law holds, and which gives a high degree of accuracy with a minimum of computational work. This method is applied here to certain ternary mixtures of the four lowest nitroparaffins, and it can be generalized so as to apply to mixtures of any number of components.

Various experimental factors, which must be considered in accurate analytical work, are also discussed.

Apparatus

The infrared spectrograph used was practically identical with that described by Wright (*9*). The 60° rock-salt prism had faces 10 by 8 cm. and the collimating parabolic mirror had a focal length of 91.4 cm. (36 inches). A water-cooled Nernst glower operating on a voltage-stabilized line served as the source of radiation. A Weyrich compensated vacuum thermocouple served as receiver. The primary galvanometer deflections were amplified by a balanced photovoltaic cell device. The pri-

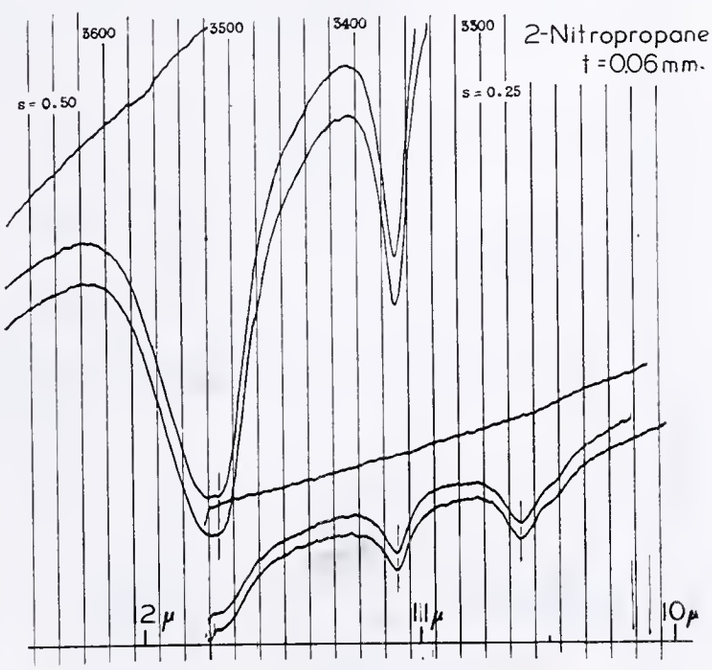

FIGURE 1. PART OF A RECORD OF THE INFRARED SPECTRUM OF 2-NITROPROPANE

Wave length region, 12.45 to 10.06μ. Upper trace recorded with no cell in radiation path, lower traces with 0.06-mm. cell in path

[1] Present address, Naval Research Laboratory, Washington, D. C.

mary galvanometer and the amplifier were mounted on a large Mueller support (*6*, *7*) placed on a basement pillar. The secondary galvanometer was mounted on a smaller Mueller support about 2.5 meters from the recording camera. The deflections could also be read on a scale.

The fixed-thickness absorption-cells and the precision variable-thickness cell used will be described elsewhere.

Infrared Absorption Spectra of Nitromethane, Nitroethane, 2-Nitropropane, and 1-Nitropropane

The four lowest nitroparaffins were selected for this work because of their commercial and scientific importance (*5*). These compounds are all similar, two of them being isomers, and their boiling point range is rather narrow, necessitating lengthy fractionation for complete separation.

Since the only available data on the infrared absorption spectra of the nitroparaffins were the early measurements by Coblentz (*4*) on nitromethane and nitroethane and the data of Wells and Wilson (*8*) for gaseous nitromethane, the first step in the present work was a careful investigation of the spectra of the four compounds in the liquid state. The materials, which had been purified by fractionation in a long column, were kindly supplied by the Commercial Solvents Corporation.

Figure 1 shows a portion of a record obtained with liquid 2-nitropropane. The upper curve is obtained with no absorption cell in the light path. The vertical distance between this curve and a line drawn through the zero positions gives the emission intensity, I_0, as a function of wave length. The lower curves are obtained with a cell of 0.07-mm. thickness filled with 2-nitropropane placed in front of the entrance slit. From these curves and the corresponding zero lines the intensity of the transmitted radiation, I, is determined. In Figure 2 the per cent transmission $100\ I/I_0$, has been plotted against wave length. Since these curves are used here only for choosing the wave lengths most suitable for analysis, the per cent transmission has not been corrected for background radiation nor for losses due to the cell windows.

The following characteristic bands were chosen as the most favorable for analysis: nitromethane 10.90μ, nitroethane 10.06μ, 2-nitropropane 11.74μ, 1-nitropropane 8.15μ. A glance at the curves will show that at each of these wave lengths the compound indicated has intense absorption, whereas none of the other compounds absorbs strongly. This choice allows the determination of each compound in a mixture with any of the three others. For some mixtures a different choice would permit a more sensitive analysis, but would not lend itself so well to the extension of the work to four-component mixtures. The most intense bands, those occurring around 6.5μ, cannot be used here, since they are common to all nitroparaffins.

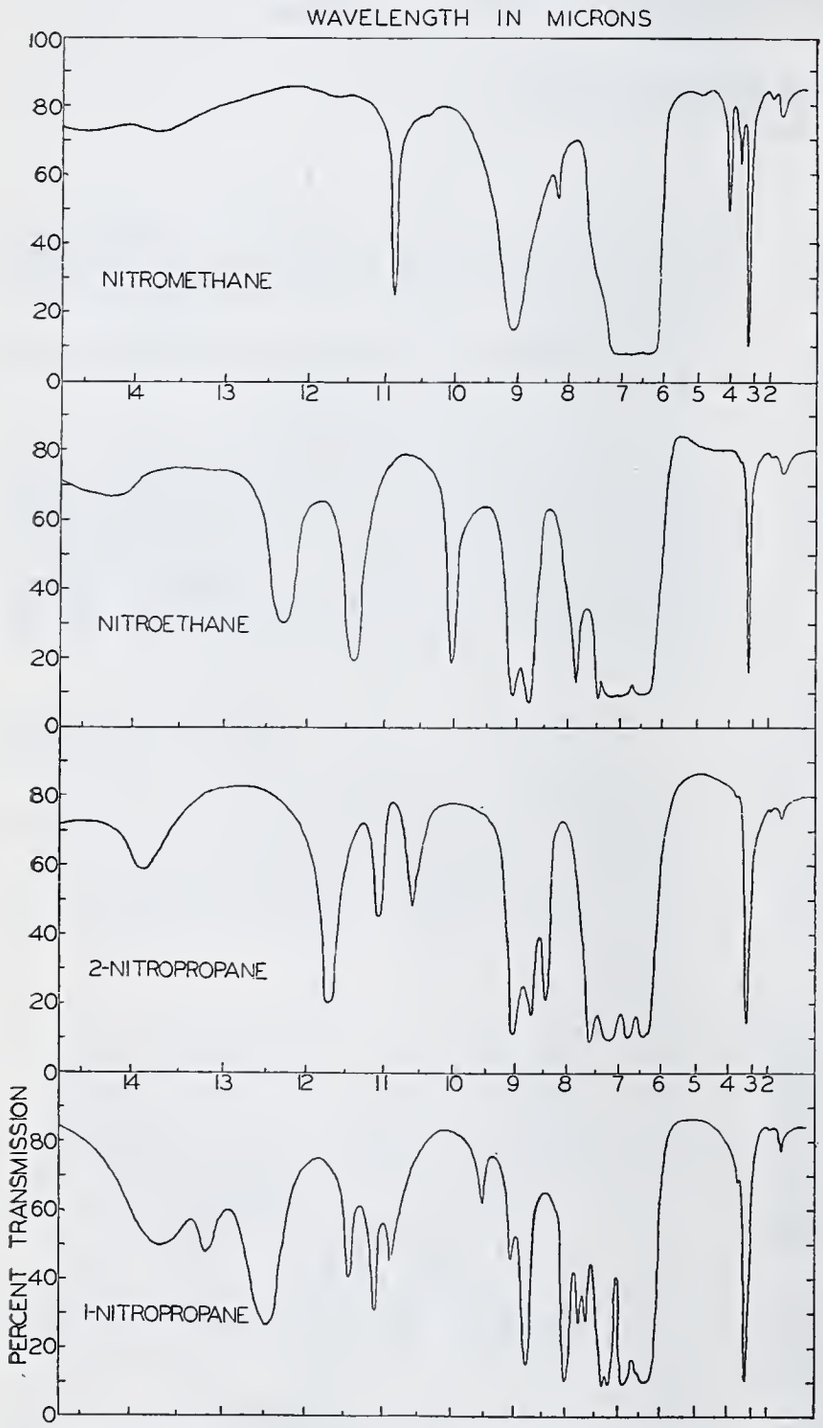

FIGURE 2. INFRARED TRANSMISSION CURVES FOR FOUR LOWEST NITROPARAFFINS

Analytical Procedure

A consideration of the boiling points suggested the following analytical problems as being of particular importance in the control of nitroparaffin production:

A. The determination of nitroethane and 2-nitropropane in nitromethane.
B. The determination of 2-nitropropane and nitromethane in nitroethane.
C. The determination of 1-nitropropane and nitroethane in 2-nitropropane.
D. The determination of 2-nitropropane in 1-nitropropane.

In each case a concentration range of the minor components from 0 to 7 per cent by volume was considered.

BINARY MIXTURES. Problem D is typical of seven problems studied. Figure 3 shows a record obtained with known solutions of 2-nitropropane in 1-nitropropane, covering a small region around 11.74μ where 2-nitropropane has a strong absorption band. Experimental results of this kind furnish the basis for all methods of infrared spectroscopic analysis, although these methods may differ considerably in the manner in which the experimental data are obtained or utilized. Common to all of the methods for analysis of binary mixtures is the use of "working curves"—i. e., plots of the ratio (or the logarithm of the ratio) of two deflections (or differences between deflections) against concentration.

'he necessary data may be en from a record such as ure 3 or may be obtained visual observation. The ailed procedure is largely natter of experimental cir- nstance and preference. ess the detecting and re- ding devices of the spec- graph are extremely stable, ingle measurement cannot trusted to be sufficiently urate. Hence, the wave- gth interval concerned must scanned several times, or instrument must be set the correct wave length several measurements en to obtain an average. e number of readings re- red is determined by the bility of the instrument and accuracy desired. It is eved that in general the st accurate readings are ob- ied by setting the instru- nt on the correct wave gth rather than by scanning, e in the latter procedure exact positions of the deflection zero may be doubtful except he ends of the region scanned. With the instrument used in present work the data obtained by averaging three or four ections with the instrument at rest were found to be more urate than readings obtained with comparable effort in other 's. This procedure was accordingly adopted. It was possible different observers to check galvanometer deflections of mm. to within 0.2 mm.

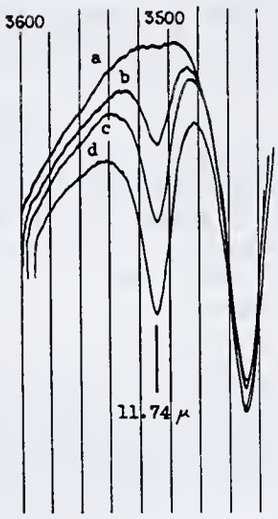

FIGURE 3. SMALL PART OF A RECORD SHOWING ABSORPTION AROUND 11.74μ OF MIXTURES OF 2-NITROPROPANE IN 1-NITROPROPANE

(a) 0.00%, (b) 2.00%, (c) 4.00%, (d) 6.00% by volume of 2-nitropropane in 1-nitropropane. Cell thickness 0.071 mm.

Vorking curves for two binary mixtures are shown in Figure 4 which the extinction, log I_0/I, for a suitably chosen wave gth is plotted against the concentration of the minor com- ent. The extinction is preferred over other functions of the ection ratio, since when the solutions obey Beer's law a ight line is obtained which minimizes considerably the k required for determining and checking the working curve. equires only about 5 minutes to fill the absorption cell and asure its extinction. On a routine basis the instrument can set on the correct wave length and the straight-line working ve checked at two points in about 15 minutes, and the analy- then follow at the rate of about ten per hour. The working ve should be checked occasionally, since it may shift owing to nges in transmission of the absorption cell with deterioration he rock-salt faces, accumulation of dirt, etc. For these and eral other binary mixtures investigated the error of the analy- never exceeded 0.1 per cent of total sample. The smaller con- rations could have been measured more accurately with a of greater thickness; the range of measurable concentrations ld have been increased by using a thinner cell.

ERNARY MIXTURES. The analysis of a mixture having two or components is made in three steps: (1) the presence of one or component is neglected, and the approximate concentra- of the other minor component is determined by the procedure a binary mixture; (2) the approximate concentration of the ected component is then determined in a similar manner; , finally, (3) the approximate concentrations of the two minor ponents are corrected for the error caused by the neglected ponent in each case.

ne first chooses a wave length, λ′, at which minor component as strong absorption and the other two components have small absorption. A working curve is made for a binary mixture of component 1 in the major component at this wave length, the extinction of the ternary mixture is measured, and the approximate value $\bar{c}_1$ for the concentration of component 1 is determined from this working curve. The more nearly equal the absorption of component 2 and the major component are, the more nearly correct will $\bar{c}_1$ be.

A wave length, λ″, is chosen at which component 2 has strong absorption and component 1 and the major component have weak absorption. A working curve for component 2 is made and the approximate concentration $\bar{c}_2$ of component 2 in the mixture is determined.

Finally, the corrected concentrations c_1 and c_2 are determined by

$$c_1 = \bar{c}_1 - \frac{\epsilon_2' - \epsilon_0'}{\epsilon_1' - \epsilon_0'}\bar{c}_2 \qquad (1)$$

$$c_2 = \bar{c}_2 - \frac{\epsilon_1'' - \epsilon_0''}{\epsilon_2'' - \epsilon_0''}\bar{c}_1$$

where ϵ_0', ϵ_1', and ϵ_2' are the extinction coefficients at the wave length, λ′, of the major component and of minor components 1 and 2, respectively; and ϵ_0'', ϵ_1'', and ϵ_2'' are the extinction coefficients at λ″. Equations 1 are derived in the next paragraph.

TABLE I. DETERMINATION OF 2-NITROPROPANE AND NITROETHANE IN NITROMETHANE

Mixture		c_{2-NP}	c_{NE}
		% by volume	
A-1		7.01	3.07
		7.01	2.97
		7.13	3.07
		7.02	3.19
		6.99	2.97
	Av.	7.03	3.05
	"Correct"	7.00	3.00
A-2		5.03	5.10
		4.96	5.07
		5.25	4.93
		5.20	5.01
		4.99	4.89
	Av.	5.08	5.00
	"Correct"	5.00	5.00
A-3		2.96	6.99
		3.06	7.09
		3.22	7.03
		2.94	7.12
		2.92	6.87
	Av	3.02	7.02
	"Correct"	3.00	7.00

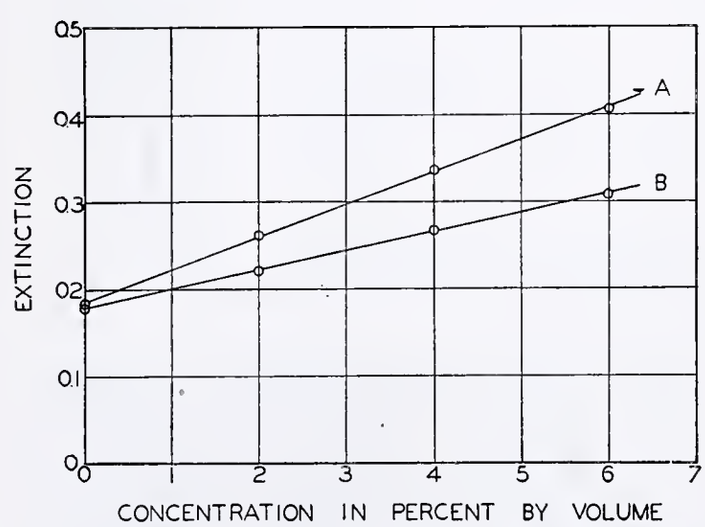

FIGURE 4. WORKING CURVES

A. For determination of 2-nitroparaffin in 1-nitroparaffin. Wave length 11.74μ. Slit 0.60 mm. Cell thickness 0.071 mm.
B. For determination of 1-nitroparaffin in 2-nitroparaffin. Wave length 8.15μ. Slit 0.25 mm. Cell thickness 0.071 mm.

The small factors $(\epsilon_2' - \epsilon_0')/(\epsilon_1' - \epsilon_0')$ and $(\epsilon_1'' - \epsilon_0'')/(\epsilon_2'' - \epsilon_0'')$ need be determined only once. The former may be conveniently obtained by dividing the slope of the working curve, already made, for determining component 1 in the major component into the slope of a working curve, made with the same cell and at the same wave length, λ', for determining component 2 in the major component. The other correction factor may be determined similarly.

Table II. Determination of Nitromethane and 2-Nitropropane in Nitroethane

Mixture		c_{NM}	c_{2-NP}
		% by volume	
B-1		7.11	3.00
		7.15	3.23
		7.15	3.23
		7.09	3.11
		6.98	3.18
	Av.	7.10	3.15
	"Correct"	7.00	3.00
B-2		5.13	5.25
		5.32	5.40
		5.32	5.27
		5.15	5.26
		5.25	5.19
	Av.	5.23	5.27
	"Correct"	5.00	5.00
B-3		2.98	7.21
		3.25	7.20
		3.26	7.20
		2.93	6.91
		2.90	7.08
	Av.	3.06	7.12
	"Correct"	3.00	7.00

In Tables I, II, and III are given the results of five entirely independent analyses of each of eight ternary mixtures. The symbols A-1, A-2, etc., designate the different mixtures. The symbols c_{NM}, c_{NE}, c_{1-NP}, and c_{2-NP} are used to designate the concentrations of nitromethane, nitroethane, 1-nitropropane, and 2-nitropropane, respectively.

Table III. Determination of 1-Nitropropane and Nitroethane in 2-Nitropropane

Mixture		c_{1-NP}	c_{NE}
		% by volume	
C-1		4.03	2.01
		3.85	1.81
		3.92	2.12
		4.11	2.12
		3.96	1.96
	Av.	3.97	2.01
	"Correct"	4.00	2.00
C-2		1.88	3.95
		1.89	3.96
		1.89	3.88
		2.01	3.84
		1.94	3.93
	Av.	1.92	3.91
	"Correct"	2.00	4.00

While 26 of the 80 measured concentrations deviate from the "correct" values by 0.15 per cent by volume or more, only 9 of the observed concentrations deviate from the average by that amount. This indicates that some of the values listed as "correct" are somewhat in error. If all 80 measurements are considered, and if the fact that they are not all measurements of the same quantity is neglected, an over-all standard deviation for a single measurement of 0.093 per cent by volume is obtained.

The time required to determine the working curves was about 45 minutes, and each analysis took about 15 minutes. In routine analysis the working curves would need to be redetermined only occasionally, say, after sets of ten or twenty analyses.

Theoretical

Beer's law states that the absorption of each component of a mixture is independent of the presence of the other components or, more precisely, that the extinction suffered by a beam of monochromatic light on passing through a layer of the mixture is a linear function of the concentrations of the individual components.

Let Figure 5 represent an absorption cell filled with a liquid mixture. Let I_0 represent the intensity of the incident radiation, I_0' the intensity of the radiation transmitted by the front window, I' the intensity of the radiation having passed through the liquid, and I the intensity of the radiation transmitted by the filled cell. If the liquid mixture obeys Beer's law,

$$I' = I_0' \times 10^{-(\epsilon_0 c_0 + \epsilon_1 c_1 + \epsilon_2 c_2 + \ldots\ldots) l} \qquad (2)$$

where l is the thickness of the liquid, $c_0, c_1, c_2, \ldots$ are the concentrations of the individual components of the mixture, and $\epsilon_0, \epsilon_1, \epsilon_2, \ldots$ are their extinction coefficients. If the concentrations are expressed in moles per liter and l in centimeters, the ϵ's are called molecular extinction coefficients. In this paper the concentrations will be expressed in per cent by volume.

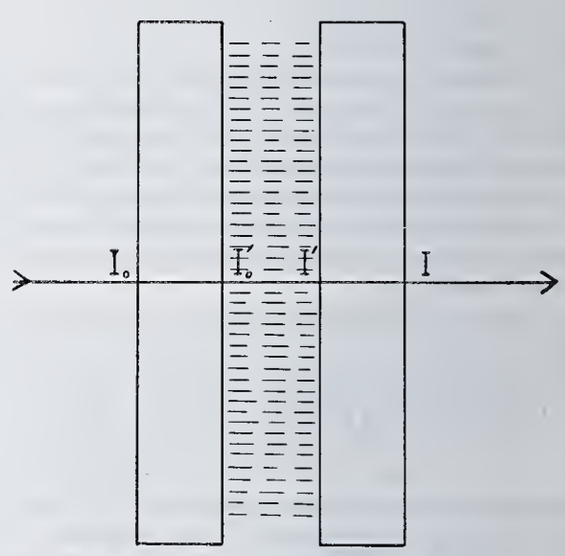

Figure 5. Absorption Cell (Schematic)

Definition of I_0, I_0', I', and I

Substitution of the relations

$$I_0' = T_1 I_0$$

and

$$I' = I/T_2$$

where T_1 and T_2 are the transmission factors for the front and back windows, into Equation 2 and taking logarithms gives

$$\log (I_0/I) = (\epsilon_0 c_0 + \epsilon_1 c_1 + \epsilon_2 c_2 + \ldots\ldots) l - \log T_1 - \log T_2$$

or, with obvious abbreviations,

$$E = (\epsilon_0 c_0 + \epsilon_1 c_1 + \epsilon_2 c_2 + \ldots\ldots) l + K \qquad (3)$$

We shall call Equation 3 the "cell equation". E is the extinction for the given wave length used, and K is a positive quantity which will be called the "cell constant", since it depends largely upon the nature and condition of the windows of the cell. K varies in a gradual manner with the wave length. The extinction coefficients, $\epsilon_0, \epsilon_1, \epsilon_2, \ldots\ldots$, on the other hand, are rapidly varying functions of the wave length, which are different for different com-

pounds. To characterize them unambiguously it is necessary to specify the resolution of the instrument as well as the temperature of the absorbing liquid. For most liquids the extinction coefficients are nearly constant over a temperature range of a few degrees.

The working curves shown in Figure 4, and all the other working curves obtained for binary mixtures of the nitroparaffins, are straight within the experimental error. Thus, it may be concluded that mixtures of the nitroparaffins obey Beer's law with considerable accuracy in the infrared. In fact, these working curves may be regarded as graphs of the cell equation

$$E = (\epsilon_1 - \epsilon_0)c_1 l + 100\,\epsilon_0 l + K \qquad (4)$$

obtained by substituting $c_0 = 100 - c_1$, $c_2 = c_3 = \ldots\ldots = 0$, in Equation 3.

From Equation 4 it is seen that the sensitivity of the analysis increases with the difference between the extinction coefficients of the two components and also with the cell thickness. The difference in extinction coefficients depends upon the choice of wave length, and ϵ_0 limits the value of l which can be used without making E too large to be measured accurately. Hence, in choosing the wave length for an analysis it is important to keep ϵ_0 small and $(\epsilon_1 - \epsilon_0)$ large. If the validity of Beer's law has been established for a mixture, only two points are required to determine the working curve. If the same cell is always used so that l is constant, the slope remains constant, and the working curve need be checked at only one point for shifts due to changes in K.

In view of the relation $c_0 + c_1 + c_2 = 100$ per cent by volume, the analysis of a ternary mixture requires extinction measurements at only two wave lengths, λ' and λ''. They are chosen in such a manner that ϵ_1 is large while ϵ_2 and ϵ_0 are small at λ', whereas ϵ_2 is large and ϵ_1 and ϵ_0 are small at λ''. If the extinctions and the cell constants have been measured at these wave lengths, two cell equations can be written down. Marking the quantities measured at λ' and λ'' by single and double primes, respectively, the solution of these equations is

$$c_1 = \frac{(E' - K' - 100\,\epsilon_0' l)(\epsilon_2'' - \epsilon_0'') - (E'' - K'' - 100\,\epsilon_0'' l)(\epsilon_2' - \epsilon_0')}{(\epsilon_1' - \epsilon_0')(\epsilon_2'' - \epsilon_0'')l - (\epsilon_1'' - \epsilon_0'')(\epsilon_2' - \epsilon_0')l} \qquad (5)$$

and an analogous expression for c_2.

If the very slight dependence of the cell constant upon the composition of the mixture is neglected, $K' + 100\,\epsilon_0' l$ is equal to E_0', the extinction of the cell filled with the major component; also,

$$\epsilon_2'' - \epsilon_0'' = \frac{1}{100\,l}(K'' + 100\,\epsilon_2'' l - K'' - 100\,\epsilon_0'' l) = \frac{1}{100\,l}(E_2'' - E_0'')$$

Substitution of these and the analogous relations into the expressions for c_1 and c_2 gives

$$c_1 = 100\,\frac{(E' - E_0')(E_2'' - E_0'') - (E'' - E_0'')(E_2' - E_0')}{(E_1' - E_0')(E_2'' - E_0'') - (E_1'' - E_0'')(E_2' - E_0')} \qquad (6)$$

and an expression for c_2 which may be obtained by interchanging subscripts 1 and 2.

It is essential to choose wave lengths λ' and λ'' in such a manner that, with a given inaccuracy in the extinction measurements, the inaccuracy in the computed concentrations will be as small as possible. A simple analysis, which will not be given here, shows that one should strive to satisfy the following conditions: $(\epsilon_1' - \epsilon_0')^2 + (\epsilon_2' - \epsilon_0')^2$ and $(\epsilon_1'' - \epsilon_0'')^2 + (\epsilon_2'' - \epsilon_0'')^2$ should be as large as possible, and $(\epsilon_1' - \epsilon_0')(\epsilon_1'' - \epsilon_0'') + (\epsilon_2'' - \epsilon_0'')(\epsilon_2' - \epsilon_0')$ should be as small, numerically, as possible. When the previously described procedure for selecting the wave lengths is used, these conditions tend to be fulfilled. The expressions for the concentrations may then be simplified as follows:

$$c_1 = 100\,\frac{(E' - E_0')(E_2'' - E_0'') - (E'' - E_0'')(E_2' - E_0')}{(E_1' - E_0')(E_2'' - E_0'')\left\{1 - \dfrac{(E_2' - E_0')(E_1'' - E_0'')}{(E_1' - E_0')(E_2'' - E_0'')}\right\}}$$

$$\cong 100\,\frac{E' - E_0'}{E_1' - E_0'} - 100\,\frac{E'' - E_0''}{E_2'' - E_0''} \times \frac{E_2' - E_0'}{E_1' - E_0'}$$

or

$$c_1 = \bar{c}_1 - \frac{\epsilon_2' - \epsilon_0'}{\epsilon_1' - \epsilon_0'}\,\bar{c}_2 \qquad (7)$$

where $\bar{c}_1$ and $\bar{c}_2$ are the values for the concentrations obtained from the measurements of E' and E'' by means of the two-component working curves. Similarly,

$$c_2 = \bar{c}_2 - \frac{\epsilon_1'' - \epsilon_0''}{\epsilon_2'' - \epsilon_0''}\,\bar{c}_1 \qquad (8)$$

The relative error introduced by using these approximation formulas is:

$$\frac{(E_2' - E_0')(E_1'' - E_0'')}{(E_1' - E_0')(E_2'' - E_0'')} = \frac{(\epsilon_2' - \epsilon_0')(\epsilon_1'' - \epsilon_0'')}{(\epsilon_1' - \epsilon_0')(\epsilon_2'' - \epsilon_0'')}$$

The value of this quantity was about 0.0009 for Problem B. Thus, the systematic error introduced by the simplification was less than 0.1 per cent of concentration c_1. In the two other cases of analysis of ternary mixtures of the nitroparaffins this error was even smaller. In all these cases this systematic error was negligible compared to the inaccuracy caused by the errors of observation.

The error introduced by the simplification leading to Equations 7 and 8, when expressed as a percentage of the concentration to be determined, is independent of this concentration. Hence, the method outlined here is not limited to mixtures for which the concentrations of the components to be determined are small

Background Radiation

The existence of stray radiation inside the spectrograph is revealed by the fact that the measured intensity, I, differs from zero for wave lengths for which the absorption is virtually complete, as indicated by the flattening of the bands. This may be seen in the transmission curves of Figure 2 for the strong absorption bands at about 6.5μ. Even when a glass shutter was used it was found necessary to correct I_0 and I for background radiation whenever the extinction data were used for determining absolute values of extinction coefficients, cell constants, cell thicknesses, etc.

The background correction for I_0 was determined by using a very thin cell with highly polished windows filled with carbon tetrachloride, which has strong absorption bands near the wave lengths of interest but almost no absorption in the short wave length region. The residual intensity, measured at a wave length for which the absorption is virtually complete and multiplied by a factor (about 1.1) to compensate for the reflection losses in the cell, gives the correction which must be subtracted from the measured value of I_0. When expressed as a percentage of I_0, this correction can be applied to any later value of I_0.

The background correction for I must be determined for the particular cell with which measurements are being made, and, strictly, for the cell filled with the liquid being studied. If the liquid has a band giving complete absorption near the desired wave length, the residual deflection at this band is taken to be the correction.

If there are intense bands near the wave length in question, the residual deflections at these bands are determined, and the desired correction is found by interpolation. For very thin cells, where the absorption for no band is complete, more elaborate methods must be employed.

The magnitude of the background correction is indicated by the following examples: The correction for I was 5.4 per cent of

I_0 at 11.74μ (slit width 0.60 mm.), 3.8 per cent of I_0 at 10.06μ (slit 0.50 mm.), and 2.3 per cent of I_0 at 8.15μ (slit 0.25 mm.). The corrections for I_0 were slightly larger.

Determination of Cell Constant

The constant, K, for a given cell at a given wave length may be determined by filling the cell with a liquid which has negligible absorption at this wave length and measuring I_0 and I. For, when $\epsilon = 0$, the cell equation (9) becomes $E = \log (I_0/I) = K$.

If no liquid is available which is transparent at the desired wave length, one or more liquids which are transparent at other wave lengths may be used to determine K for these wave lengths, and the cell constant for the desired wave length may then be obtained by interpolation.

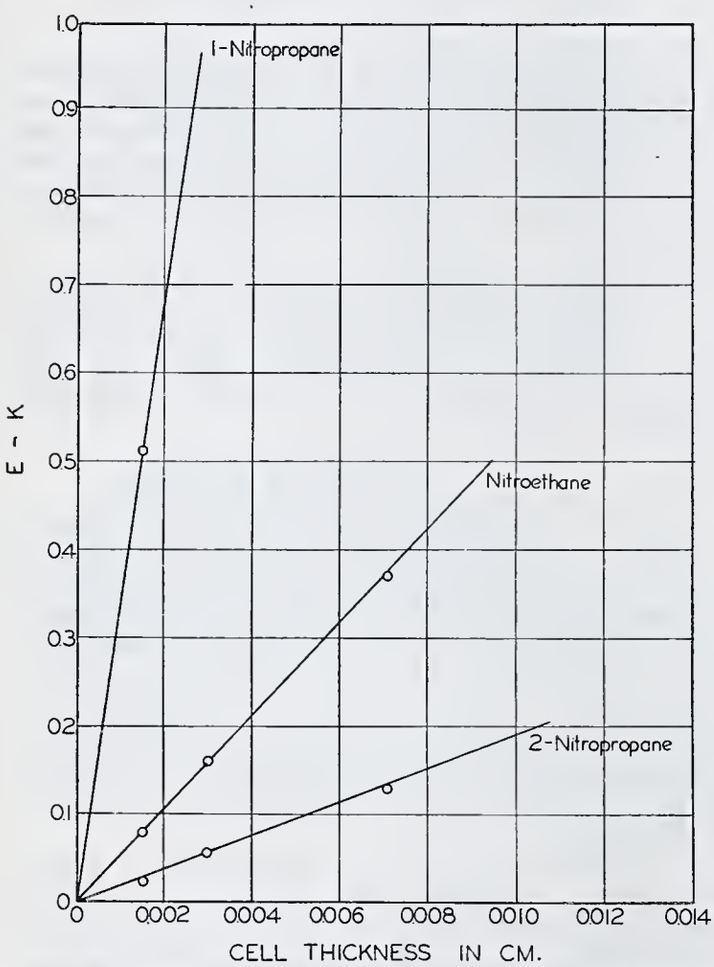

FIGURE 6. DETERMINATION OF EXTINCTION COEFFICIENTS AT 8.15μ WITH CONSTANT-THICKNESS CELLS

Slit width 0.25 mm. Extinction coefficients, measured by slopes of lines, are: 2-nitropropane 0.178, nitroethane 0.533, 1-nitropropane 3.30 cm.$^{-1}$ × (per cent by volume)$^{-1}$

If a liquid is available whose extinction coefficient is known for the given wave length, and if the cell thickness is known, the cell constant may be determined by measuring E and applying Equation 9. The liquid or liquids used for determining K should strictly have the same index of refraction at the desired wave length as the liquid under investigation. However, the variation in refraction index is rarely large enough to require consideration.

Measurement of Cell Thickness

For thick cells the thickness of the spacer can be measured with vernier calipers before the cell is assembled. Thinner cells must be measured after they are assembled. The following method was found satisfactory for all cells which could not be measured before assembly.

The thickness of a 10.0-mm. cell was measured with calipers to within 0.1 per cent. The cell was then filled with standard solutions of pure n-hexane in pure carbon tetrachloride, and the extinction coefficient of n-hexane at 3.41μ was determined. The thin cell to be measured was then filled with standard solutions of n-hexane in carbon tetrachloride of sufficient concentration to give accurately measurable E-values. The extinctions and the cell constant were measured at 3.41μ, and $E - K$ was plotted against the concentration of n-hexane. The cell thickness was then determined by dividing the slope of the curve by the extinction coefficient of n-hexane. In this manner the thickness of thin cells could be determined to within 1 per cent or better.

Determination of Extinction Coefficients

Since the extinction coefficient of an absorbing compound is independent of the amount of sample used, and since it is of fundamental importance in the analysis of mixtures obeying Beer's law, its use for specifying absorption is to be preferred to the more widely used term of per cent transmission. Various methods of measuring extinction coefficients have been investigated.

The cell equation for a cell filled with a pure compound is

$$E = \log (I_0/I) = \epsilon cl + K \qquad (9)$$

Hence, if the thickness of the cell is accurately known, measurement of E and K enables one to determine ϵ. However, since high and low values of E cannot be measured accurately, the data obtained with a single cell do not give accurate values of ϵ over the entire wave-length range but only for those regions where the absorption gives intermediate values of E for the particular cell. To obtain accurate values over a wide wave-length range, a set of cells of varying thickness is required. All intensity measurements must be corrected for background, and the values of K and l must be accurately determined.

For the wave lengths selected for analytical work increased accuracy was obtained by averaging the extinction coefficient values obtained with cells of different thickness. $E-K$ was plotted against l and ϵ was determined by measuring the slope of the straight line and dividing it by the concentration. Figure 6 shows the curves obtained for nitroethane, 1-nitropropane, and 2-nitropropane at 8.15μ (slit 0.25 mm.). Only for the thickest cell could the background correction be determined directly by filling the cell with 1-nitropropane, which has a strong absorption band at 8.15μ. The background corrections for the thinner cells were estimated by comparing their transmissions with that of the thickest cell at some wave length where the liquid does not absorb. For 1-nitropropane the absorption at 8.15μ was so strong that only with the thinnest cell was the extinction small enough to be measurable.

TABLE IV. EXTINCTION COEFFICIENTS

Wave Length *Microns*	Slit Width *Mm.*	Nitromethane	Nitroethane	2-Nitropropane	1-Nitropropane
		(*Cm.* × *% by volume*)$^{-1}$			
8.15	0.25	0.252	0.486	0.177	3.38
10.06	0.50	0.097	1.85	0.063	0.061
10.90	0.55	2.09	0.114	0.135	0.782
11.74	0.60	0.035	0.328	(5.26)	0.194

The results show that it is possible to determine extinction coefficients fairly accurately with cells of fixed thickness. The procedures worked out for measuring the cell thickness, the cell constant, and the background correction are satisfactory. However, the use of a set of several cells to obtain accurate extinction coefficients for analytical work is laborious and cumbersome, and high accuracy is not easily obtained for large extinction coefficients. For these reasons another method which employs a variable-thickness cell was developed.

The precision variable-thickness cell constructed will be described elsewhere. It is essentially an all-metal Baly-type cell with rock-salt windows and a screw drive. With this cell, curves similar to those of Figure 6 can be easily and quickly obtained. In addition, several simplifications occur. Since the cell constant does not change with the thickness setting, its value need not be determined. Similarly, since no appreciable time is required to change the setting of the cell, the intensity, I_0, of the incident radiation can easily be held constant while several measurements are taken and, hence, its value need not be determined.

The cell equation may be written

$$-\log I = \epsilon cl + K - \log I_0 \qquad (10)$$

and, hence, the extinction coefficient can be determined from a plot of log I *vs.* l. Such plots are shown in Figure 7. For this purpose the absolute thickness of the cell is of no importance; only the thickness increments between successive cell settings are required, and these can be determined with higher accuracy than the absolute thickness values.

In Table IV are listed the extinction coefficients for the four nitroparaffins for each of the four wave lengths chosen for analytical work. These values were used to obtain the correction factors used in the ternary analyses reported. The coefficients are defined by the convention used here of expressing the concentrations in per cent by volume. The molecular extinction coefficients, obtained by multiplying these values by the molecular weight divided by ten times the density, are listed in Table V.

Table V. Molecular Extinction Coefficients

Wave Length *Microns*	Slit Width *Mm.*	Nitromethane	Nitroethane	2-Nitropropane	1-Nitropropane
8.15	0.25	1.35	3.47	1.59	30.0
10.06	0.50	0.52	13.2	0.57	0.54
10.90	0.55	1.12	0.82	1.21	6.95
11.74	0.60	0.19	2.34	(47.2)	1.72

The extinction coefficient for 2-nitropropane at 11.74μ was so large that it could not be determined directly. The following procedure was therefore used. The difference between the extinction coefficients for 2-nitropropane and nitromethane was first determined by using known binary mixtures, and the extinction coefficient for nitromethane was then added to this difference.

The accuracy obtained with the variable-thickness cell depends upon three factors: the precision with which the thickness increments are determined, the accuracy of the intensity measurements, and the accuracy of the background correction. The first two quantities were measured with less than 1 per cent error. It is not easy to estimate the accuracy with which the background correction was made. However, the fact that the plots of log I *vs.* l are all very nearly linear indicates that the background correction applied to the intensity values was not much in error.

The values, 0.178 and 0.177, for the extinction coefficient of 2-nitropropane at 8.15μ measured with fixed-thickness cells and the variable-thickness cell, respectively, are in excellent agreement. For nitromethane and 1-nitropropane the agreement is not so close. The values obtained by the variable-thickness cell should be the more accurate.

Extinction coefficient data enable one to predict accurately the sensitivity which will be obtained when a given wave length is used in a binary analysis and, hence, to choose the optimum wave length and the optimum cell thickness for any particular analysis without additional experimental work; as a matter of fact, since the slope of the working curve is determined by the differences between the extinction coefficients, and since the intercept of the curve is given by the extinction of the cell filled with the pure solvent, the working curve can be drawn without recourse to standard solutions, if Beer's law is known to hold. The correction factors used in the simplified method of analysis of ternary and multicomponent mixtures are also determined by extinction coefficients. Hence, the use of a variable-thickness cell, with which extinction coefficients can so easily be measured, represents an important advance in analytical work with mixtures obeying Beer's law.

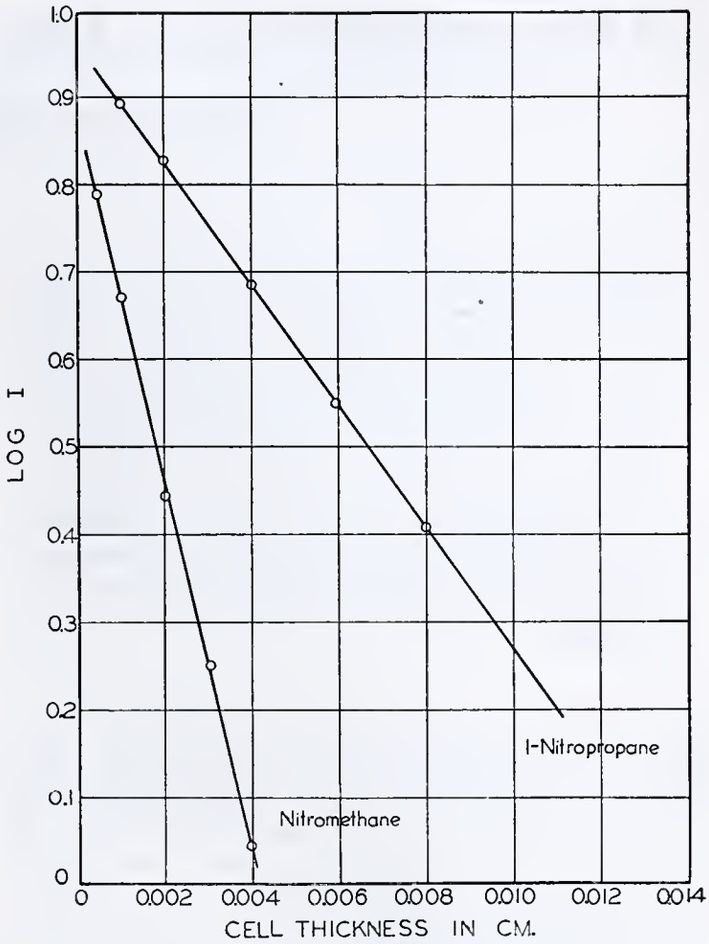

Figure 7. Determination of Extinction Coefficients at 10.90μ with the Variable-Thickness Cell

Slit width 0.55 mm. Extinction coefficients, measured by negative slopes, are nitromethane 2.09, 1-nitropane 0.782 cm.$^{-1}$ × (per cent by volume)$^{-1}$

Acknowledgments

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Determination of the pH of Textile Materials

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In all previously published methods for the determination of the pH of leather, paper, and textiles, a single-extraction operation has been used on the assumption that the amount of water per unit weight of sample is relatively insignificant. The results of the present investigation, however, show that for textiles the pH of the extract solution is a function of the quantity of water used in the extraction process. An extrapolation method based on this fact is described for the determination of the pH of a fabric. The pH of a textile is defined as the pH of the water present in the cloth under A. S. T. M. standard conditions of temperature and humidity.

STUDIES by Kolsky and Jones (*7*) and by Crist (*4*) have called attention to the importance of hydrogen-ion concentration in textiles as a factor affecting color stability, tensile strength, and other fabric properties. Their work has emphasized the need for a general understanding of what is meant by the pH of a cloth and the desirability of a uniform and reliable method of measurement.

The U. S. Army Quartermaster Corps has included a pH requirement in a recent tentative specification for barracks bag material (*9*), proposing a method somewhat similar to the A. S. T. M. standard method for determining the pH of scoured wool and of cotton tape (*1*). Both these methods are based on a single-extraction operation, using 75 to 250 ml. of water or buffer solution and 2 to 10 grams of sample, and measurement of the pH of the extract solution, either colorimetrically or potentiometrically. The value thus obtained is taken as the pH of the textile material. In general, these methods are similar to those now in use for the determination of the pH of paper and leather (*2, 8, 14, 15*).

The differences in procedures proposed by the various investigators indicate the need for an adequate definition of pH for a substance of low moisture content such as cloth. The purpose of the present investigation is to set up such a definition and to examine the single-extraction method in the light of that definition.

Definition

Applying the equation pH = minus log $[H^+]$, in which $[H^+]$ is the molar hydrogen-ion concentration in a water solution, the definition of the pH of a fabric must be based on the moisture content of the fabric. In extraction, the water in contact with the textile will dissolve out the minute amounts of acids, bases, or buffer salts contained therein. The pH of the extract solution will be determined by the concentration of these soluble substances and, therefore, by the amount of water used for the extraction. The smaller the amount of water used, the more accurately the pH obtained represents the pH of the original air-dried fabric.

The nature of this effect of concentration on pH can be shown by simple measurements of acid, base, and buffer salt solutions.

One-tenth mole portions of reagent-grade ammonium chloride, ammonium acetate, sodium acetate, potassium carbonate, and oxalic acid were weighed out and dissolved in five 10-ml. portions of distilled water. Using a Beckman Model G pH meter with the standard 6.25-cm. (2.5-inch) electrodes furnished with the instrument, the pH values of these solutions were measured at this and conveniently higher dilutions. The results thus obtained were plotted against the dilution in milliliters per millimole of dissolved chemical.

The resulting experimental curves, shown in Figure 1, indicate that the shape of the pH curve varies with the type of chemical dissolved and that the pH observed at high dilution may be appreciably different from that of the concentrated solutions. The limiting value of the pH as the concentration increases is that of the saturated solution. These results are in accord with theoretical calculations.

Under the standard A. S. T. M. conditions (*3*) of 21° C. and 65 per cent relative humidity, various textiles may contain up to about 20 per cent of moisture by weight (*6, 10, 11, 12*). It is apparent from the curves in Figure 1 that the pH of a fabric must be obtained by an extrapolation to a concentration corresponding to the amount of water present in the cloth, and that therefore any precise definition of the pH of a fabric should take into account the effect of humidity. With this in mind, the pH of a textile may be logically defined in terms of the hydrogen-ion concentration of the water present in the cloth under the A. S. T. M. standard conditions.

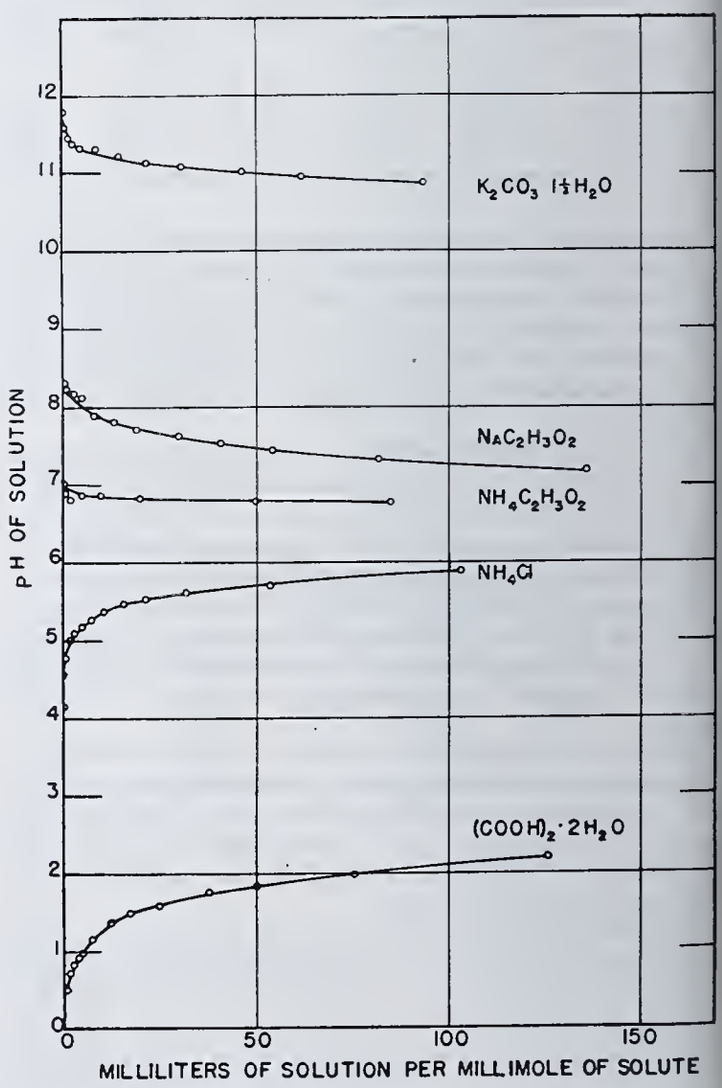

FIGURE 1. EFFECT OF CONCENTRATION ON pH OF SOLUTIONS

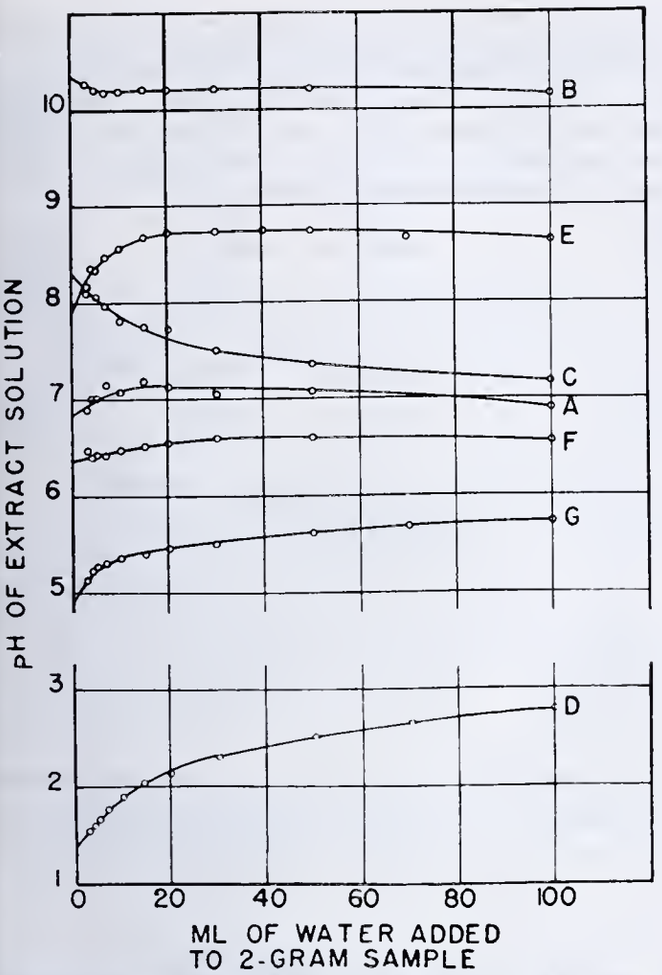

FIGURE 2. EXPERIMENTAL CURVES ON VARIOUSLY TREATED CLOTH SAMPLES

A. Cotton twill without further treatment.
B. Cotton twill treated with dilute potassium carbonate solution and air-dried.
C. Cotton twill treated with dilute sodium acetate solution and air-dried.
D. Cotton twill treated with dilute oxalic acid solution and air-dried.
E. Cotton twill dyed with a sulfur color, washed with hot water, and air-dried without further aftertreatment.
F. Cotton twill dyed with a sulfur color and aftertreated with a bath containing acetic acid, copper sulfate, and sodium dichromate, then washed and air-dried.
G. 8.5-ounce jute burlap, 12 × 12 yarn count, used in the Southern Regional Research Laboratory for studies in mildew-proofing.

Textile pH used in this sense should not be confused with total acidity, which may be obtained by titration of acid or base present.

Goode and Cox (*5*) have described a micro glass electrode that measured textile pH values for very low moisture concentrations. Their method appears to measure the pH of cloth as defined above more closely than any other published procedure, but it fails, nevertheless, to determine the true pH of the cloth under actual conditions of use.

Method of Measurement

Measurements of the pH of textiles are being made in the Southern Regional Research Laboratory by the extrapolation method described below.

A 2-gram sample of the fabric to be tested is weighed and cut into small pieces, which are placed in a small beaker and wet with 3 ml. of boiled distilled water. The fabric pieces are macerated with a spatula until the mass is uniformly wet. [Von Bergen and Crowley (*13*) of the Forstmann Woolen Co. have found from a series of experiments performed on wool, that for successful application of the method, the fabric must be wettable by the water.] The beaker is covered with a watch glass and allowed to stand for about 30 minutes, after which the mixture is again stirred. The cloth is then pressed against the side of the beaker until about 1 ml. of water has been squeezed out. This is poured into the 5-ml. cup of the Beckman pH meter, and the pH is measured on the standard 2.5-inch electrodes of the meter. Since in making determinations in neutral or nearly neutral mixtures some time is required for the absorbed gases or minute amounts of acids or bases from previous measurements on the electrodes to be replaced by the new solution, the meter indicator must be allowed to come to equilibrium and the electrodes should be carefully rinsed with distilled water after each measurement.

When the pH has been measured, the solution is returned to the beaker and another milliliter of water stirred into it. This mixture is allowed to stand for 15 minutes, then is stirred and squeezed to yield a more dilute solution for another pH measurement. In this manner pH measurements are obtained for water-cloth mixtures containing 3, 4, 5, 7, 10, 15, 20, 30, 50, and 100 ml. of water. The pH values of these dilutions are plotted against the volume of water added and extrapolated back to the zero ordinate—that is, to the air-dried condition—to give the pH of the fabric as defined earlier. In actual practice it has been found practical to make measurements of dilutions with 3, 5, 10, 20, and 30 ml. of water only, since these values are usually sufficient for the extrapolation.

Precautions against the usual errors in pH measurement are taken throughout each experiment. The pH of the water should be frequently checked to guard against contamination from acid or alkaline vapors in the air of the room. The pH meter should be calibrated periodically with standard buffer solutions and the accuracy of potentiometric measurement determined by checking against a standard cell.

The typical curves shown in Figure 2 were obtained in the above manner from a number of cloth samples. All the cotton samples were from 36-inch, 96 by 64, 2.85 cotton twill that had been prepared for dyeing by a regular caustic boil and bleaching process. Samples were given the several treatments indicated in Figure 2.

Examination of the curves will show that only in some cases does the pH of the extract liquid equal the extrapolated pH value. The assumption that the pH of the goods will be the same as the pH of an extract solution appears not to be valid for certain textile samples and thus cannot be relied upon as the basis of any general method of measurement. Curves *C* and *E*, for example, show nearly identical extrapolation values but widely different pH values at the 100-ml. concentrations. Attempts to measure relative hydrogen-ion concentrations by comparing extract solutions obtained with some standard quantity of water would, therefore, lead to erroneous results.

From the curves in Figures 1 and 2 it is apparent that as the solutions become more dilute the pH approaches that of pure water. The curve for sample *E* in the region below 30 ml. is an exception to this generality; the pH here rises farther above 7 with greater dilution. This abnormality seems to be typical of a number of sulfur-dyed cotton fabrics measured in this laboratory. No explanation for this behavior is offered.

TABLE I. REPRODUCIBILITY OF MEASUREMENTS ON SAMPLES OF UNTREATED SULFUR-DYED COTTON, *E*

Sample	pH at 5 Ml.	Deviation from Mean	pH at 10 Ml.	Deviation from Mean
1	8.21	0.06	8.52	0.01
2	8.28	0.01	8.49	0.02
3	8.23	0.04	8.43	0.08
4	8.25	0.02	8.43	0.08
5	8.38	0.11	8.50	0.01
6	8.19	0.08	8.53	0.02
7	8.29	0.02	8.53	0.02
8	8.32	0.05	8.61	0.10
9	8.33	0.06	8.57	0.06
10	8.23	0.04	8.48	0.01
Mean	8.27		8.51	
Average deviation from mean		0.05		0.04

In order to test the reproducibility of measurement with a particular sample, 10 samples of the untreated sulfur-dyed cotton (*E*) were treated with 5 ml. and then with 10 ml. of water, pH readings being taken at each dilution. The results of these

measurements, along with deviations from the mean and the average deviation from the mean value, are shown in Table I.

The value obtained at the 5-ml. point for a single observation is, therefore, subject to an average error of 0.05, and this error would increase somewhat for smaller amounts of water. For a given set of observations the average error of measurement involved in the extrapolation would be of the order of 0.1 pH unit. Greater accuracy may be obtained by averaging several measurements on similar samples.

Some inaccuracy in extrapolation is encountered when the curvature of the plot at higher concentrations is fairly pronounced. As was pointed out in connection with Figure 1, however, the extreme value of pH attainable by extrapolation would be that of a saturated solution.

Time is not a critical factor in measurements of the pH, since equilibrium between the water and the fabric is usually attained rather rapidly. All extractions were made at room temperature because a hot extraction may result in decomposition of materials in the cloth and thus lead to abnormal values.

Discussion

Using the method described, a large number of pH measurements have been made in this laboratory on cloth samples under prevailing atmospheric conditions. It is apparent from the results of this investigation that the pH of a fabric depends upon its moisture content, which, in turn, is a function of the relative humidity and temperature of the surrounding atmosphere. In setting up specifications and tests, therefore, it would seem essential that a precise definition including the cloth conditions be adhered to. In order that a definite value may be assigned to it, the pH of a fabric or textile material might be defined as the pH of the water present under A. S. T. M. standard conditions of 21° C. and 65 per cent relative humidity. This value is the one which would be obtained by the proposed extrapolation method using cloth samples with a moisture content brought to equilibrium in a standard conditioning room.

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Photometric Estimation of Silicon in Magnesium and Magnesium Alloys

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In a rapid and accurate method for the determination of small amounts of silicon in magnesium metal and magnesium alloys, use is made of the molybdenum blue color developed from sodium sulfite reduction of the silicomolybdic acid complex at a pH range of 7.0 to 7.3. Interfering weak bases are complexed by the use of ammonium tartrate after color development.

THE necessity for the determination of small amounts of silicon in magnesium metal and its alloys has become increasingly urgent. According to Mellor (*8*), silicon and silica are converted to silicides in molten magnesium and appear as a eutectic mixture. Occasionally, with larger amounts of silicon, crystals of Mg_2Si form, according to Beck (*2*), which may be detected metallographically. Examination of the hydrochloric acid residue from 100 grams of incendiary bomb alloy revealed no free silicon. In view of these findings, it is assumed that all silicon in magnesium metal is present as silicide which may be readily converted to silica by treatment with nitric acid.

This work is intended to describe a rapid and accurate photometric method for the determination of silicon in magnesium and its alloys. It is based on the reduction of the silicomolybdic acid complex $H_8[Si(MoO_7)_6].H_2O$ (*3*, *6*) to molybdenum blue, $Mo_3O_8.H_2O$ (*9*).

The first attempt in this laboratory to determine the amount of silicon in magnesium metal and its alloys made use of the yellow complex (*10*, *11*). Iron, which offered the principal interference in this procedure, was removed as basic formate (*12*). Following this practice, the silicon found was consistently low.

A procedure for the determination of silica has been reported utilizing the blue color from a sodium sulfite reduction of the silicomolybdic acid complex (*4*, *5*, *7*). In order to prevent the hydrolysis of salts of aluminum, zinc, and iron present in the solution of the alloys, the molybdenum blue was first developed by sulfite at a pH of 3, using formic acid to attain this acidity. As in the case of acetic acid (*7*) continued reduction of the ammonium molybdate reagent yielded utterly unreliable results. At this pH, microscopic bubbles of sulfur dioxide very definitely interfere with transmission readings.

To make possible the comparison of the molybdenum blue on the neutral or alkaline side, ammonium tartrate was employed to complex the aluminum, zinc, and iron present in the alloys. This procedure gave consistent results. The cloudiness due to the initial hydrolysis of the salts of weak bases during the development of molybdenum blue disappeared completely at room temperature after 50 minutes. Longer periods of standing resulted in extremely small changes in transmission. In the higher concentrations of silicon the development time had greater significance. This was not sufficient, however, to warrant a development period longer than one hour.

Reagents and samples were prepared in paraffin-lined flasks and quartz beakers, respectively. Vycor glassware was also found

Table I. Addition of Silicon as Sodium Silicate

Weight of Sample	Silicon: Present by analysis	Silicon: Added	Silicon: Found	Silicon
Grams	*Mg.*	*Mg.*	*Mg.*	%
To Weighed Amounts of Magnesium Metal				
2.0000	0.062	0.051	0.126	0.0031
2.0000	0.072	0.051	0.121	0.0036
2.0000	0.074	0.051	0.124	0.0037
2.0000	0.080	0.051	0.128	0.0040
2.0000	0.099	0.051	0.143	0.0050
To Weighed Amounts of Magnesium Alloys Containing Zinc, Aluminum, and Manganese				
1.2756	0.402	0.100	0.502	0.0315
1.0884	0.294	0.100	0.405	0.0267
1.0584	0.397	0.100	0.513	0.0375
1.5240	0.431	0.100	0.438	0.0283

Table II. Influence of Copper on Standard Sodium Silicate Solution

Silicon Present	Copper Present	Silicon Found
Mg.	*Mg.*	*Mg.*
0.625	1	0.640
0.625	2	0.610
0.625	3	0.578
0.625	4	0.575
0.625	None	0.625

to be suitable. The only contact with less resistant glassware came at the time of making the sample up to volume and in comparison in the colorimeter cell.

Color measurements were made on a Lumetron colorimeter, Model 402 E, using a narrow-band filter having maximum transmission at 610 millimicrons, which effectively eliminated the error due to iron. If this element is present in amounts no greater than 0.02 per cent, any visual or photometric instrument is adequate for the estimation of silicon. The 50-mm. depth cell was employed. A Beckman pH meter was used for all pH adjustments.

Aluminum, iron, nickel, zinc, lead, tin, manganese, and titanium occurring in amounts characteristic of magnesium alloys offered no interference.

Copper exceeding 0.2 per cent resulted in slightly lower transmission values. This error did not increase markedly with larger amounts of copper. Alloys containing 0.1 to 0.2 per cent copper were dissolved in cold 6 *N* sulfuric acid. The copper was removed by filtration and the filtrate boiled with nitric acid to complete the oxidation of the silicon compounds. Small losses of silicon hydride resulted from following this procedure.

Chloride, sulfate, perchlorate, and nitrate were without influence. Phosphate very definitely interfered, but only in concentration greater than was found in a large number of samples of magnesium and magnesium alloys.

Reagents

Hydrochloric acid, 1 *N*. Ammonium carbonate, dilute solution. Ammonium molybdate, 100 grams (81.0 per cent MoO_3) per liter. Sodium sulfite, 170 grams of anhydrous sodium sulfite per liter. Sodium silicate, 0.025 mg. of silicon per ml., standardized by dehydration with perchloric acid followed by sulfuric acid and hydrofluoric acid treatment. Ammonium tartrate, 400 grams per liter. Nitric acid, equal volumes of concentrated acid and water.

Procedure for Determining Silicon in Magnesium Metal and Magnesium Alloys

Dissolve 2 grams of the metal in a quartz or Vycor beaker by the dropwise addition of 50 ml. of 1 to 1 nitric acid. Boil for 15 minutes to remove all oxides of nitrogen. Cool, transfer to a 100-ml. volumetric flask, and make up to the mark. Pipet a 25-ml. aliquot into a quartz or Vycor beaker, adjust to a pH of approximately 4 with ammonium carbonate, add 5 ml. of ammonium molybdate reagent, and adjust with the hydrochloric acid reagent to a pH range of 2.4 to 2.7. Allow the silicomolybdic acid complex to develop for 2 minutes. Add 25 ml. of sodium sulfite reagent. After letting stand for 10 minutes, add 10 ml. of ammonium tartrate reagent and dilute to 100 ml. Read the transmission at about 610 millimicrons in the colorimeter after a 50-minute period of standing.

If copper is present in amounts greater than 0.2 per cent, dissolve the sample in a quartz or Vycor beaker by the dropwise addition of cold 6 *N* sulfuric acid. Filter off the copper residue, add 10 ml. of concentrated nitric acid, and boil, proceeding as above.

If fluoride is present, error due to this element may be avoided by the addition of 5 ml. of saturated boric acid prior to solution of the sample in nitric acid (*1*).

Aliquots containing no more than 0.05 mg. of silicon were used to establish the results in Tables I to IV.

Summary

Silicon in magnesium alloys may be determined colorimetrically by the molybdenum blue method with an average error of 5 per cent on amounts ranging from 0.002 to 0.05 per cent. Probably higher concentrations might be determined with the same degree of accuracy, provided sufficient care were maintained in making dilutions. The maximum amount of silicon that may be present in the final aliquot should not exceed 0.05 mg. Use of ammonium tartrate prevented the interference of aluminum, iron, and zinc, making possible the measurement of a stable molybdenum blue at a pH of approximately 7.2.

Copper concentrations up to 0.2 per cent gave no interference. Error due to larger amounts of copper was eliminated by solution of the alloy in cold 6 *N* sulfuric acid with subsequent filtration and oxidation of the soluble silicon hydride with nitric acid. Lead, manganese, tin, titanium, and nickel, in concentrations normally encountered in magnesium alloys, did not interfere.

Table III. Influence of Phosphorus on Standard Sodium Silicate Solution

PO_4	Silicon Present	Silicon Found
Mg.	*Mg.*	*Mg.*
0.0	0.125	0.127
0.05	0.125	0.131
0.10	0.125	0.127
0.15	0.125	0.129
0.20	0.125	0.134
0.30	0.125	0.126
1.35	0.125	0.116

Table IV. Influence on Sulfuric Acid Solution of Magnesium Alloy Containing Zinc, Manganese, and Aluminum

Weight of Alloy	Silicon Found, Solution in Sulfuric Acid	Silicon Present, Solution in Nitric Acid
Gram	*Mg.*	*Mg.*
1.0000	0.265	0.283
1.0000	0.258	0.283

Acknowledgment

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Colorimetric Determination of Fluoride in Natural Waters with Thorium and Alizarin

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A THORIUM-alizarin lake has been used, following isolation of fluoride by distillation, in the determination of fluoride by volumetric (*3*) and colorimetric (*3*) procedures. In the method described herein this lake has been adapted to the colorimetric determination of small quantities of fluoride in potable waters, without prior isolation by distillation, in a manner similar to the use of a zirconium lake in many of the colorimetric methods (*1*, *4*, *5*) now in use. The use of thorium overcomes several of the difficulties experienced with the use of zirconium—i. e., slowness of the reaction, tendency of the lake to precipitate, and instability of the reagents.

The method involves a combination of hydrolytic, adsorption, and complex-ion equilibria. A dilute solution of thorium nitrate at pH 3.5 consists of a hydrous thorium dioxide sol stabilized by the preferential adsorption of hydrogen and thorium ions (*7*). Being positively charged in this condition, the oxide further adsorbs anions which in this case are nitrate ions. Alizarin monosodium sulfonate is a salt of a weak acid and, consequently, is almost completely hydrolyzed at pH 3.5 to the yellow associated acid. The acid, however, dissociates (*2*) in the presence of thoria sol with the formation of a deep red adsorption complex of alizarin sulfonate ion and the positively charged hydrous oxide. This process is principally an exchange adsorption of the alizarin sulfonate ion with less strongly adsorbed anions on the oxide. Addition of fluoride to the adsorption complex converts the thoria into a fluoride of thorium, releasing alizarin sulfonate ions which reassociate into the yellow alizarin sulfonic acid. With reagents of proper concentrations, the color changes progressively from red to yellow with increasing quantities of fluoride. Thus a series of colorimetric standards may be prepared with which a sample of unknown fluoride content may be compared.

Equilibrium in the thoria sol is displaced by slight changes in hydrogen-ion concentration; therefore, the pH of both sample and standards must be stabilized by means of a buffer. The system sodium formate–formic acid has a pH of about 3.5 and was found convenient for the purpose. In order to keep the quantity of buffer to a minimum, the sample is subjected to a preliminary neutralization. Sodium sulfate, added to both sample and standards, reduces interference of the sulfate ion.

Reagents

Thorium reagent, 0.001 *M* with respect to thorium nitrate, and 1 *M* with respect to each sodium sulfate, formic acid, and sodium formate.

Alizarin indicator, 0.00025 *M*, 0.0855 gram of alizarin monosodium sulfonate in 1 liter.

Standard fluoride solution, 1 ml. equals 0.01 mg. of fluoride; 0.0221 gram of sodium fluoride in 1 liter.

Nitric acid, 0.3 *N*.

Procedure

Standards. Transfer 0, 2, 4, 6, 8, 10, and 12 ml. of standard fluoride solution to a series of 100-ml. long-form Nessler tubes, each of which has been previously marked at 110 ml., and add 5 ml. of alizarin indicator to each. Dilute to 110 ml. with distilled water and add 5 ml. of thorium reagent. Mix well and allow to stand 30 minutes.

Sample. Transfer a 100-ml. sample to an Erlenmeyer flask and add to it 5 ml. of alizarin indicator. Titrate carefully with 0.3 *N* nitric acid to a pure yellow. Pour into a Nessler tube, dilute to 110 ml., add 5 ml. of thorium reagent, and mix well. Compare after 30 minutes with the standards. Should the fluoride content of the sample be beyond the range of the standards, repeat the determination with a smaller sample.

Effects of Ions and Conditions

Common Ions. Experience with zirconium-alizarin methods (*1*, *4*, *5*) and the method described has shown that, when corrections are made only for the effects of sulfate and chloride, the fluoride found in a 100-ml. sample is less than double or quadruple the fluoride found in a 50-ml. or 25-ml. sample diluted to 100 ml. This indicates the necessity of considering the combined interference of other ions tending to give low results whose individual effects may be negligible. Although the effects of the interfering ions are not strictly additive, the error introduced in applying corrections on the assumption that they are additive is of no consequence in the determination of fluoride in a sample containing no more than 100 mg. of any one ion. Approximate effects of the common ions are as follows: 100 mg. of sulfate are equivalent to +0.02 mg. of fluoride; 100 mg. of calcium, magnesium, or chloride are equivalent to −0.01 mg. of fluoride; 100 mg. of nitrate are equivalent to −0.005 mg. of fluoride. Since the ionic weight of nitrate is approximately that of bicarbonate, the quantity of nitrate introduced in neutralization of the sample may be considered identical to the quantity of bicarbonate originally present. Sodium and potassium have no effect.

Other Ions. When phosphate or aluminum is present, the fluoride must be isolated by a distillation procedure (*8*) prior to determination of fluoride. Phosphate may be recognized by the turbidity of the sample caused by the precipitation of thorium phosphate. Aluminum is indicated by failure to obtain the pure yellow color of the alizarin when the sample is neutralized with nitric acid. Aluminum forms a lake with alizarin, the orange color of which is not immediately discharged by an excess of the acid. Iron occasionally may be present in sufficient quantities to give a purple or black tinge to the sample, making comparison difficult. In such case iron may be removed from the water sample by aerating and filtering. Acid waters should be made alkaline before aerating. Silica in quantities as high as 20 mg. does not interfere.

Color. Waters containing organic coloring matter may be analyzed for fluoride by the Walpole (*6*) technique by using 100-ml. short-form Nessler tubes in a simply constructed comparator in which the sample tube rests above a tube of distilled water and the standard rests above a tube of a duplicate sample containing 5 ml. of thorium reagent but no alizarin. Comparison is made by viewing from the top through the lengths of two tubes.

Temperature. Differences of temperature between standard and sample cause an apparent deviation of 0.002 mg. of fluoride per degree centigrade. The standards and samples are preferably brought to constant temperature in a water bath.

Time. Equilibrium is reached within 30 minutes. No visible change occurs in the standards upon standing as long as a week in darkness.

Summary

A procedure is proposed for the colorimetric determination of oride in natural waters containing not more than 1000 parts per lion of any one of the common ions. By applying small cortions, results may be obtained to 0.1 part per million of fluoride h the use of a 100-ml. sample. Phosphate and aluminum erfere, but their presence may be recognized in the course of determination. The method excels previous colorimetric thods in speed, accuracy, and stability of reagents.

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Modified Basic Succinate Estimation of Aluminum in Magnesium Alloys

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The modification of the Willard and Tang basic succinate procedure described is rapid, simple, and accurate and is advocated as a routine as well as a referee method for aluminum. The accuracy obtained is equivalent to, or, in certain cases, greater than, that obtained with the benzoate-oxine procedure. Procedures are given for avoiding interference from iron, silicon, and copper.

THE purpose of this investigation is to establish a rapid and accurate routine method for the determination of aluminum magnesium alloys and to compare it with the recent benzoate-ne method (*2*), which involves preliminary separation of minum as benzoate from most bivalent metals.

The basic succinate method submitted is a modification of the llard and Tang procedure (*4*) for the determination of alunum by precipitation with urea. In the modified procedure as d by the laboratories of Basic Magnesium, Incorporated, the oy is dissolved in dilute hydrochloric acid, to which ammonium oride, succinic acid, and urea are subsequently added. This ution, diluted to the proper volume, is made just alkaline to thyl orange by the addition of dilute, freshly filtered amnium hydroxide or a solution of ammonium carbonate. iling the solution gently for 90 minutes results in the antitative precipitation of aluminum as basic succinate. This ocedure for aluminum is believed by the authors to be the most id accurate method available. It requires only a modicum analytical skill and is advocated as a routine as well as referee thod to establish the aluminum content of magnesium alloys. e precipitate of basic aluminum succinate is extremely dense, that filtration is rapid. One precipitation is sufficient for ective separation from bivalent metals commonly occurring in gnesium alloys. This separation is not quantitatively possible the ammonium hydroxide procedure for aluminum, particuly in the presence of zinc (*1*).

Reagents

Succinate reagent. 10 grams of urea, 5 grams of ammonium oride, and 5 grams of succinic acid, diluted to 300 ml.

Hydrochloric acid, c. p.; perchloric acid, c. p.; ammonium droxide, filtered; ammonium carbonate, c. p., 5 per cent solution. Phenylhydrazine, c. p.; hydroxylamine hydrochloride, c. p.; and ammonium bisulfite, 10 per cent solution.

Procedure

Weigh a 1-gram sample of the alloy into a 600-ml. beaker. Treat with 50 ml. of distilled water and 10 ml. of concentrated hydrochloric acid. After the alloy is dissolved, filter to remove metallic copper. The interference due to appreciable amounts of iron is prevented by adding to the heated filtrate a few drops of a 10 per cent solution of ammonium bisulfite and 2 ml. of phenylhydrazine. This treatment serves to reduce the iron and to maintain it in a ferrous state during the precipitation of aluminum as basic succinate. Dilute with 300 ml. of succinate reagent and boil gently. Make the solution just alkaline to methyl orange with freshly filtered 1 to 1 ammonium hydroxide or a solution of ammonium carbonate. Continue boiling gently for 90 minutes, precipitating the aluminum as basic succinate. Gentle boiling is very effective and does not reduce the total volume quickly. No special care need be given the solution during this period.

Filter through a paper of loose texture. Dissolve the small amount of precipitate which adheres to the beaker in 20 ml. of 1 to 4 hydrochloric acid and reprecipitate by adding 1 to 1 ammonium hydroxide until just alkaline to methyl red. Boil the solution for one minute and filter through the paper containing the major portion of the precipitate. Wash the precipitate six times with hot 1 per cent ammonium chloride made alkaline to methyl red with ammonium hydroxide. Ignite the precipitate at 1300° C. for one hour to form nonhygroscopic Corundum, cool, and weigh as oxide. Unglazed porcelain crucibles appear to be best for this purpose since they show no change in weight. For long life of the crucible it is advisable to dry the crucible and precipitate in a standard oven before ignition. For rapid routine analysis, it is preferable to brush the precipitate out of the crucible and weigh directly as oxide.

If silicon is present in quantities greater than 0.2 per cent dissolve the alloy in 30 ml. of 1 to 2 perchloric acid. Evaporate the solution on a hot plate to copious fumes of perchloric acid. Cool, dilute to 50 ml. with distilled water, and filter through a fast paper. Wash the precipitate six times with hot distilled water and discard. Copper is dissolved by this procedure. In order to eliminate the error due to this element, add 20 ml. of the ammonium bisulfite reagent to the solution (*4*), or (if iron is absent) 1 gram of hydroxylamine. Add 300 ml. of the succinate reagent and continue as described previously.

Discussion

For a comparison of the basic succinate method with the benzoate-oxine method and with precipitation by ammonium carbonate (used because it is silica-free), a standard solution of aluminum chloride was prepared by dissolving aluminum metal

Table I. Analyses of Standard Solution of Aluminum Chloride

Aluminum Determination	Al Present	Al Found	Error	pH of Filtrate
	Mg.	*Mg.*	*Mg.*	
As oxine by benzoate-oxine precipitation	51.55	51.28	−0.27	5.3
	51.55	51.30	−0.25	5.3
	51.55	51.49	−0.06	5.5
As oxide by ammonium carbonate precipitation	51.55	51.32	−0.23	7.1
	51.55	51.43	−0.12	6.8
	51.55	51.48	−0.07	6.8
As oxide by basic succinate precipitation	51.55	51.59	+0.04	5.2
	51.55	51.59	+0.04	5.2

Table II. Analyses of Standard Solution of Aluminum Chloride Containing Magnesium, Nickel, Manganese, Cadmium, and Iron

Aluminum Determination	Al Present	Al Found	Error
	Mg.	*Mg.*	*Mg.*
As oxine by benzoate-oxine precipitation	51.80	52.32	+0.52
	51.80	52.21	+0.41
	51.80	52.25	+0.45
As oxide by basic succinate precipitation	51.80	51.80	0.0
	51.80	51.75	−0.05
	51.80	51.86	+0.06

of 99.99 per cent purity in a sufficient quantity of dilute hydrochloric acid. This was diluted to 1 liter, from which 25-ml. aliquots were taken containing 51.55 mg. of aluminum as chloride. A second solution was later prepared containing 51.80 mg. of aluminum as chloride per 25 ml. of solution.

To each of 6 samples of aluminum chloride solution containing 51.80 mg. of aluminum, the following substances were added as chlorides: 1 gram of magnesium, 5 mg. each of nickel, manganese, and cadmium, and 2 mg. of iron.

As indicated in Table II, the samples were analyzed for aluminum by both the benzoate-oxine and modified basic succinate procedures. The interference of iron was avoided in the precipitation of aluminum as basic succinate with ammonium bisulfite–phenylhydrazine treatment, as described in the Procedure. The bisulfite-phenylhydrazine treatment was used in various unsuccessful attempts to reduce the iron error in the benzoate-oxine method. Stenger, Kramer, and Beshgetoor (*2*) suggest the removal of iron by treatment with hydrogen sulfide before precipitation of aluminum with oxine. This refinement was not made. Since the concentrations of contaminants present in these six samples greatly exceed those normally found in magnesium alloys, the figures in Table II should not be taken as typical of benzoate-oxine results on ordinary alloys. They do show, however, that no interference occurs in the basic succinate procedure.

Table III. Analyses of Standard Solution of Aluminum Chloride Containing Magnesium, Aluminum, and Zinc

Aluminum Determination	Series	Al Present	Al Found	Error
		Mg.	*Mg.*	*Mg.*
As oxide by benzoate precipitation (ignition temperature 1300° C.)	A	51.80	52.54	+0.74
	B	51.80	52.70	+0.90
As oxide by basic succinate precipitation	A	51.80	51.75	−0.05
	B	51.80	51.86	+0.06

In order to establish the relative tendencies to coprecipitate zinc in the determination of aluminum by both the basic succinate and benzoate procedures, the latter not followed by oxine precipitation, synthetic solutions were prepared containing magnesium, aluminum, and zinc. Series A contained one gram of magnesium, 51.80 mg. of aluminum, and 40 mg. of zinc. Series B contained the same concentrations of magnesium and aluminum with 80 mg. of zinc.

Table IV illustrates the error introduced due to silica contamination in the aluminum precipitation by both benzoate and succinate procedures. Each sample contained 51.80 mg. of aluminum as chloride. The aluminum precipitates were analyzed colorimetrically for silica.

Table IV. Error Due to Silica

Aluminium Precipitation	SiO_2 Added as Sodium Silicate	SiO_2 Found in Precipitate
	Mg.	*Mg.*
As benzoate	1.0	0.045
	2.5	0.254
	5.0	0.468
By succinate	1.0	0.000
	2.5	0.000
	5.0	0.027

In a few instances magnesium alloys may contain as much as 0.5 per cent silicon. Chloride solutions were prepared containing 1 gram of magnesium, 50 mg. of aluminum, and 5.5 mg. of silica as sodium silicate. Dehydration by the use of perchloric acid (*3*) resulted in the recovery of the silicon without measurable loss of aluminum from solution. Hydrofluoric-sulfuric acid treatment of the insoluble residue from perchloric acid dehydration is unnecessary for the amounts of silicon usually present in magnesium alloys.

Table V. Duplicate Analyses of Magnesium Alloys Containing Zinc (3%), Aluminum (6%), and Manganese (0.3%)

Sample	Al by Succinate	Al by Benzoate-Oxine
	%	%
1	5.98	6.00
1a	5.98	5.99
2	5.36	5.32
2a	5.36	5.35
3	5.94	5.93
3a	5.94	5.95
4	5.76	5.82
4a	5.73	5.83
5	5.71	5.85
5a	5.70	5.72

Summary

A modification of the Willard and Tang basic succinate procedure is advocated as a rapid and accurate routine method as well as a referee method for aluminum. The accuracy attained is equivalent to, or, in certain cases, greater than, that given by the benzoate-oxine procedure. One precipitation is sufficient to separate aluminum effectively as basic succinate from most bivalent metals. The resulting precipitate is extremely dense so that filtration and washing are rapid. It is essential to ignite the precipitate at 1300° C. in order to convert it completely to nonhygroscopic Corundum within one hour. For this purpose a Hevi-Duty muffle, Model HDT 5610, was used. A temperature of 1200° C. may be used if a sufficiently long ignition period is allowed. Procedures are given for avoiding interference from iron, silicon, and copper.

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Iodometric Estimation of Zinc in Magnesium Alloys

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By modification of Lang's iodometric procedure, zinc may be determined in the presence of iron, after removal of manganese by persulfate oxidation and of copper as free copper by boiling with test lead. A direct procedure is outlined for alloys containing more than 2 per cent of zinc, and a concentration method employing hydrogen sulfide is used for alloys containing less than this amount.

THE increasing demand for magnesium alloys containing aluminum and zinc has stimulated investigation of an iodometric method for the determination of zinc as proposed by Lang (*2, 3*) and later by Gamzulov (*1*). Modification of the original procedure makes possible the determination of zinc in the presence of iron, after the removal of manganese as manganese dioxide by persulfate oxidation, and of copper as free copper by boiling with test lead.

The general principle of the Lang method involves the liberation of iodine by the addition of potassium ferricyanide to a slightly acid zinc sulfate solution containing excess potassium iodide. The zinc precipitates as $K_2Zn_3[Fe(CN)_6]_2$ according to the equation:

$$ZnSO_4 + 2K_3Fe(CN)_6 + 2KI = K_2Zn_3[Fe(CN)_6]_2 + 3K_2SO_4 + I_2$$

$$2Fe(CN)_6^{---} + 2I^- \rightleftharpoons 2Fe(CN)_6^{----} + I_2$$

The iodine liberated is titrated with standard sodium thiosulfate. After the precipitation of zinc is complete, the rate of liberation of iodine from potassium iodide by potassium ferricyanide becomes sufficiently retarded to make possible an extremely accurate estimation of zinc.

The method is advocated for magnesium alloys containing zinc in excess of 2 per cent. The inconvenience of precipitating zinc as the sulfide and hydrogen sulfide gas is eliminated, making possible the determination of a larger number of samples in much less time. The accuracy obtained is equal to or greater than that of the conventional methods for zinc.

If alloys of lower zinc composition are encountered, it is necessary to concentrate the zinc from a larger sample by a preliminary hydrogen sulfide separation, or to add standard zinc sulfate solution to the sample before titration.

Reagents

Potassium ferricyanide, C. P. (0.2 molar); test lead, granular; ammonium persulfate, C. P.; sodium or ammonium citrate, C. P. (10 per cent solution). Manganous sulfate, C. P. (1 gram of manganese per liter of solution); potassium iodide, C. P.; starch solution (1 per cent); sodium thiosulfate, 0.1 *N*, and hydrogen sulfide.

Procedure

Transfer 2 grams of the alloy to a 400-ml. beaker, and add 10 ml. of distilled water and 35 ml. of 1 to 2 sulfuric acid in small portions. During this operation keep the beaker covered as much as possible with a watch glass to avoid loss by mechanical spray. In the event copper is present, add 5 to 10 grams of test lead and boil the solution for 15 minutes. Filter through a test paper and wash the residue five times with hot distilled water. To the filtrate, add sufficient manganous sulfate solution to ensure the presence of approximately 10 mg. of manganese in the sample. Add not more than 1 gram of ammonium persulfate and boil the solution for 30 minutes. The manganous sulfate which is added ensures the precipitation of all manganese present as a filterable manganese dioxide. Filter the hot solution through a Selax No. 3001 or similar porcelain filtering crucible into a 500-ml. Pyrex suction flask. Wash thoroughly, controlling the final volume to not more than 150 ml. Add 10 ml. of 10 per cent sodium or ammonium citrate solution for each milligram of iron present in the sample. Add ammonium hydroxide dropwise to the alkaline color change of methyl orange. Usually a turbidity occurs at this point. Occasionally the indicator is oxidized and more must be added. Add 1 to 2 sulfuric acid until the solution is distinctly acid to methyl orange. The volume at this point should not exceed 200 ml. Place the flask in a cooling bath to bring the solution to room temperature. Add 1 to 2 grams of potassium iodide and let stand for 10 to 15 minutes. Add 2 or 3 ml. of 1 per cent starch solution.

To discharge the blue coloration due to iodine liberation which may develop, add sodium thiosulfate, making certain that no excess is introduced. Add potassium ferricyanide reagent in 1- to 2-ml. portions and titrate slowly with standard sodium thiosulfate after each addition until the starch-iodine color is faint. Continue this procedure until no additional color is developed on addition of potassium ferricyanide reagent. A few more drops of sodium thiosulfate solution will discharge the remaining iodine and complete the titration. A sulfur-yellow end point is obtained which is sharp to within one drop of 0.1 *N* sodium thiosulfate. The end point should persist for at least 30 seconds, although in most instances it remains much longer. Upon standing, however, the potassium ferricyanide reagent liberates iodine slowly, bringing back the blue color. A light green end point indicates the presence of copper or iron that has not been completely removed or complexed.

In the event the alloy contains only a few tenths of a per cent of zinc, the following hydrogen sulfide procedure is used:

Weigh a 10-gram sample of the alloy into a 600-ml. beaker, dissolve the sample in 100 ml. of 1 to 1 sulfuric acid, and dilute the solution to 300 ml. Neutralize the excess acid with ammonium hydroxide. Add 6 *N* sulfuric acid dropwise until just acid to methyl orange. Pass hydrogen sulfide through the solution at a rate of at least eight bubbles per second for a period of 45 minutes. Filter through close-textured paper. It is not necessary to wash the precipitate.

Return the filter paper and contents to the original beaker. Add 5 ml. of concentrated nitric acid and 10 ml. of 70 per cent perchloric acid. Place on a hot plate, and boil to destroy the paper, taking the solution to dense fumes of perchloric acid. Permit the solution to cool. Dilute to a volume of 100 ml. with distilled water and add 5 to 7 ml. of concentrated sulfuric acid. As an alternative procedure for the destruction of filter paper and conversion of zinc sulfide to a soluble zinc salt a combination of 5 ml. of nitric acid and 10 ml. of sulfuric acid may be used. The procedure from this point on is identical with that described above, except for the omission of the persulfate step, since no manganese is present.

TABLE I. RECOVERY OF ZINC FROM STANDARD ZINC SULFATE SOLUTIONS

Zinc in Sample	Zinc Recovered	Zinc in Sample	Zinc Recovered
Gram	*Gram*	*Gram*	*Gram*
0.0125	0.0127	0.0625	0.0627
0.0250	0.0250	0.1250	0.1254
0.0500	0.0502	0.2500	0.2507

Calculation

Each milliliter of 0.1 *N* sodium thiosulfate solution is equivalent to 9.97 mg. of zinc:

$$\frac{\text{Ml. of } 0.1\ N\ Na_2S_2O_3 \times 0.00997 \times 100}{\text{Wt. of sample}} = \%\ Zn$$

Results and Discussion

The interference of iron present in the sample may be effectively prevented by the addition of citrate. Unless this precaution is taken before the addition of potassium iodide to the solution, free iodine is liberated and the ferric iron is reduced. The resulting ferrous iron is partially reoxidized during the titration, thereby producing low values for zinc. Gamzulov (*1*) noted this reduction but did not find subsequent interference from the ferrous iron.

If copper is present, even in small amounts, it produces a green color which interferes with the true sulfur-yellow end point. If present in large amounts, copper gives high values for zinc. The test lead treatment will remove at least 10 mg. of copper satisfactorily. Granular aluminum and aluminum foil were not so effective as lead.

Manganese present in the sample leads to high values for zinc. Removal of this element as manganese dioxide by persulfate oxidation is extremely effective. Approximately 10 mg. of manganese must be present to avoid the formation of a colloidal precipitate.

Cadmium produces high results for zinc and consequently must be removed if present. Preliminary investigation indicates that it may be precipitated with 40-mesh aluminum (*4*) from a slightly acid solution. Granular aluminum also successfully removes copper if a small amount of cadmium is present.

Lang (*2*) advocates complexing cobalt with potassium cyanide and eliminating the effect of nickel by the addition of sodium potassium tartrate and aluminum sulfate. However, these elements occur in such small amounts in magnesium alloys that they do not interfere.

The sodium thiosulfate solution was standardized against weighed amounts of C. P. potassium dichromate, dried at 110° C. The empirical zinc factor of 9.97 mg. of zinc per ml. of 0.1 *N* sodium thiosulfate was employed. This is the figure found by Lang and was verified by the present work.

Results of tests to determine the accuracy of the iodometric method are shown in Table I. A standard zinc sulfate solution was prepared by dissolving freshly ignited C. P. zinc oxide in sulfuric acid. Portions of this solution were used as standard zinc samples in all tests.

Table II records the effect of various metallic ions on the recovery of zinc from solutions containing a uniform amount of zinc and varying amounts of the metals.

A series of analyses was run to test the applicability of the iodometric method to magnesium-zinc alloys. A 4-gram sample of alloy was dissolved and divided into two equal parts. The original zinc content was determined on one aliquot, and the zinc content after the addition of a standard zinc solution was determined on the other. Table III shows the results of these tests.

Table IV compares the zinc values obtained by two different methods. Twenty grams of alloy were dissolved in sulfuric acid and divided into two equal parts. Both solutions were gassed to concentrate the zinc as sulfide. The standard potassium ferrocyanide procedure, using uranium nitrate as an external indicator, was employed on one portion, the iodometric method on the other.

Table II. Determination of Zinc by Iodometric Method

(In solutions containing magnesium, aluminum, manganese, cadmium, nickel, iron, and copper as sulfates. Zinc present, 0.1250 gram)

Magnesium Present	Aluminum Present	Manganese Present	Iron Present	Copper Present	Cadmium Present	Nickel Present	Zinc Recovered
Grams	*Gram*	*Gram*	*Gram*	*Gram*	*Gram*	*Gram*	*Gram*
...	0.10	....	....	....	....	....	0.1247
...	0.25	....	....	....	....	....	0.1246
...	0.50	....	....	....	....	....	0.1247
...	....	0.004[a]	....	....	....	....	0.1245
...	....	0.004	....	....	....	....	0.1255
...	....	0.012[a]	....	....	....	....	0.1251
...	....	0.010	....	....	....	....	0.1270
...	....	0.020[a]	....	....	....	....	0.1246
...	....	0.020	....	....	....	....	0.1280
...	....	0.040[a]	....	....	....	....	0.1246
...	....	0.040	....	....	....	....	0.1296
...	....	0.100	....	....	....	....	0.1330
...	....	....	0.001[a]	....	....	....	0.1252
...	....	....	0.003[a]	....	....	....	0.1249
...	....	....	0.005[a]	....	....	....	0.1248
...	....	....	....	0.0010[a]	....	....	0.1251
...	....	....	....	0.0025[a]	....	....	0.1254
...	....	....	....	0.0050[a]	....	....	0.1253
...	....	....	....	0.0050	....	....	0.1300
...	....	....	....	....	0.02[a]	....	0.1250
...	....	....	....	....	0.02	....	0.1330
...	....	....	....	....	0.10	....	0.1860
...	....	....	....	....	....	0.001	0.1248
...	....	....	....	....	....	0.002	0.1252
...	....	....	....	....	....	0.005	0.1261
...	....	....	....	....	....	0.010	0.1293
...	....	....	....	0.005[a]	0.020[a]	....	0.1252
3.7	0.20	0.004[a]	....	....	....	....	0.1246
3.7	0.20	0.012[a]	....	....	....	....	0.1248
3.7	0.20	0.020[a]	....	....	....	....	0.1248
3.7	0.20	0.040[a]	....	....	....	....	0.1251
1.9	0.10	....	0.001[a]	....	....	....	0.1251
1.9	0.10	....	0.001[a]	....	....	....	0.1253
1.9	0.10	....	0.001[a]	....	....	....	0.1250
1.9	0.10	0.012[a]	0.001[a]	....	....	....	0.1247
1.9	0.10	0.012[a]	0.002[a]	....	....	....	0.1248
1.9	0.10	0.0 2[a]	0.003[a]	....	....	....	0.1249

[a] Element removed or complexed.

Table III. Analysis of Magnesium Alloys[a]

(Containing approximately 3% zinc, 6% aluminum, and 0.3% manganese)

Sample Weight	Zinc Found by Iodometric Method	Zinc Added as Standard Zinc Sulfate Solution	Total Zinc Recovered	Error
Grams	*Gram*	*Gram*	*Gram*	*Mg.*
2	0.0721	0.0625	0.1345	−0.1
2	0.0731	0.0625	0.1354	−0.2
2	0.0721	0.0625	0.1344	−0.2
2	0.0728	0.0625	0.1353	0.0
2	0.0743	0.0625	0.1366	−0.2
2	0.0648	0.0625	0.1274	+0.1
2	0.0693	0.0625	0.1315	−0.3
2	0.0748	0.0625	0.1371	−0.2

[a] All samples contained a small amount of copper, which was removed, and of iron, which was complexed.

Table IV. Analysis of Magnesium Alloys[a]

(Containing approximately 0.4% zinc, 4.5% aluminum, 0.3% manganese)

Zinc Found by Ferrocyanide Method	Zinc Found by Iodometric Method
%	%
0.52	0.52
0.61	0.61
0.55	0.54
0.34	0.33
0.42	0.40
0.35	0.36
0.46	0.46
0.41	0.43
0.51	0.50
0.39	0.38

[a] All samples contained a small amount of copper which was removed and of iron, which was complexed.

Summary

A method is described for the iodometric determination of zinc in magnesium alloys. A direct procedure is outlined for alloys containing greater than 2 per cent of zinc, while a concentration method employing hydrogen sulfide is described for alloys containing less than this amount. The interference of copper is prevented by the removal of this element with test lead. Manganese is removed as manganese dioxide by oxidation with ammonium persulfate. Errors due to the presence of iron are eliminated by complexing the ferric iron with sodium citrate.

The accuracy obtained by this method is equivalent to that of any standard procedure for zinc. The procedure is applicable to a wide variation of magnesium alloys containing zinc.

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Determination of Tin with Mercuric Chloride

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Tin can be determined by weighing the mercurous chloride formed on adding stannous chloride to mercuric chloride. With pure tin solutions the procedure is rapid and accurate and is applicable over a range from 0.1 to over 70 per cent tin. The present paper describes the necessary procedures as applied to concentrates, cassiterite ores, and ores containing complex tin sulfides. It is especially suited for tin in low-grade ore.

THE most common method of determining tin is that in which stannic chloride is reduced by a metal and the resulting stannous chloride is titrated with a standard solution of iodine. The reducing metal may be lead (*5*), antimony (*6*), iron (*4*), or nickel (*2*). Tin may also be precipitated with cupferron in a fluoborate solution and weighed as the oxide (*1*), a method that separates tin from lead, bismuth, copper, and antimony. Various separations of tin from other metals are listed by Hillebrand and Lundell (*3*), but only a few of these refer to combinations found in ores.

The method of determining tin described in the present paper is an application of the well-known qualitative reaction between stannous chloride and mercuric chloride, which also serves to separate tin from most other metals. The precipitated mercurous chloride is allowed to settle half an hour or more, filtered off on a Gooch crucible, washed, dried at 105° C., and weighed. Weight of mercurous chloride × 0.2514 equals weight of tin, a very favorable factor. Another good feature of the method is that the tin gives visible evidence of its presence, whereas in the volumetric method it is not always certain that only tin is being titrated. The directions that follow refer more particularly to the procedures necessary to bring different types of tin ores into solution and the subsequent preparation of stannous chloride by reduction with zinc. The tin and excess zinc are finally dissolved in hydrochloric acid and mercurous chloride is determined as described above.

Rich Concentrates of Cassiterite

The chief impurities may be oxides of iron, rutile, or tourmaline. Fuse 1 gram of 200-mesh material in a porcelain crucible with sodium pyrosulfate. One gram gives a good sample and provides enough solution for 10 duplicates. Leach the melt with 50 ml. of 1 to 19 nitric acid, rinse into a beaker containing 400 ml. of 1 to 19 nitric acid, and heat to simmering for at least one hour. This digestion reprecipitates any tin dissolved by the fusion. Not more than 0.5 mg. is lost, as shown by careful tests.

Filter off the insoluble residue and wash it with hot dilute nitric acid. Dry and ignite the filter in a 30-ml. porcelain crucible. Fill this crucible half full of sodium cyanide, mix well, and fuse gently until all the cyanide is melted and the tin is reduced to metal. A few weak explosions may occur from the carbon monoxide released, so that it is advisable to hold the crucible in tongs and gently swirl it. When the melt is quiet, heat in the full flame of the Bunsen burner for 2 minutes in order to form visible beads of tin. Cool the melt in a ring around the wall of the crucible to prevent the crucible from cracking. Leach with hot water in a casserole and carefully decant the cyanide solution without filtering into a sink which is being flushed with water. Wash three times in like manner. Dissolve the tin in about 50 ml. of hot 1 to 1 hydrochloric acid and 10 ml. of concentrated sulfuric acid with the casserole covered. Solution of the tin requires several hours' digestion at about 60° C. Cool the solution of stannous chloride, filter it into a small beaker, and reserve for a later addition. Wash the residue with 1 to 4 hydrochloric acid and catch the filtrate in a 250-ml. volumetric flask. The residue may contain about 1 per cent of the tin. Fuse it with cyanide as before. Digest the second metallic residue with the main solution of stannous chloride in the beaker, adding a little concentrated hydrochloric acid if necessary. Cool and filter this solution into the volumetric flask, washing the beaker and filter with dilute acid.

Dilute the tin solution to exactly 250 ml., shake thoroughly, and transfer 25 ml., representing 0.1 gram of sample, into a 125-ml. Erlenmeyer flask. Add 2 grams of 20-mesh zinc to the slightly inclined flask. Close the flask with a 1-hole rubber stopper fitted with a glass tube having a small outlet and two enlarged bulbs in the middle to aid in condensing steam as the flask is heated. This outfit is designed to effect reduction of the tin with exclusion of air. Zinc reduces the tin to a sponge which floats after a time. At this point add 0.3 gram more of zinc and 12 ml. of concentrated hydrochloric acid. Finally heat the flask gently until all the zinc and tin are dissolved, while maintaining a continuous outward pressure of steam. Next close the capillary outlet with another rod and rubber tube connection. Cool the flask in running water, release the vacuum, and quickly pour the stannous chloride solution into 30 ml. of mercuric chloride solution containing 1 gram of mercuric chloride, a twofold weight for 0.1 gram of tin. The beaker for precipitation may be 150-ml. size. After rinsing out all the tin solution, the volume in the beaker will be about 120 ml.

Let the precipitate settle 30 minutes or more, then filter it by suction into a prepared Gooch crucible. Wash the beaker and crucible three times with 1 to 4 hydrochloric acid, then three times with hot water. This washing removes a few milligrams of lead chloride generally derived from the zinc. Dry for half an hour at 105° C. Weigh and heat for 15 minutes further to check the weight. The drying of mercurous chloride should not be unduly prolonged:

$$\text{Per cent of Sn} = \text{weight of HgCl} \times 0.02514 \times 10 \times 100$$

Results obtained on various samples are shown in Table I. Nos. 1 to 4 are tests of the method made on Bureau of Standards Sample No. 42-C, which contains 99.99+ per cent of tin. Nos. 5 and 6, 7 and 8, and 9 and 10 represent three different concentrates run in duplicate. The agreement is satisfactory for the quantity of sample taken.

Tin in Cassiterite Ores

Digest 4 grams of the pulp on the steam bath for several hours in a platinum dish with 20 ml. of 1 to 1 sulfuric acid and 15 ml. of hydrofluoric acid. Cover the dish with a larger one. After an hour remove the cover and let the solution evaporate overnight. Heat to fumes, dilute with water, and again concentrate to fumes of sulfur trioxide. Add 40 ml. of 1 to 1 nitric acid and heat until solution is practically complete. Transfer the mixture to a 600-ml. beaker, dilute to 400 ml., and heat to simmering for an hour or more. Filter off any insoluble matter, wash with hot dilute nitric acid, and fuse the ignited residue once only with sodium cyanide as described above. Make up any stannous chloride recovered to 100 ml. and determine tin in 25 or 50 ml., according to the richness of the ore. This method will handle most sulfide material low in antimony or copper.

Typical results are shown in Table I. Nos. 11 and 12 are duplicates on a sample containing some chalcopyrite. Nos. 13 and 14, 15 and 16 are duplicates on ordinary cassiterite ores. No. 19 is a Bolivian tin concentrate, Bureau of Standards No. 137, with certified tin 56.64 per cent.

Tin in Complex Sulfides

This procedure covers concentrates of the tetrahedrite group [$4Cu_2S.(Sb,As)_2S_3$] containing some stannite ($Cu_2S.FeS.SnS_2$) or teallite ($PbS.SnS_2$).

Digest 2 grams of the finely ground sample in a covered beaker with a little concentrated nitric acid. After the reaction subsides add a little more acid and evaporate on the steam bath nearly to dryness. Add 200 ml. of water and digest until the

Table I. Determinations of Tin in Various Solutions, Concentrates, and Ores

No.	Sample Taken	Sn Found	Error	Sn
	Gram	*Gram*	*Gram*	%
1	0.0510	0.0509	−0.0001	....
2	0.0510	0.0509	−0.0001	....
3	0.0816	0.0812	−0.0004	....
4	0.1020	0.1025	+0.0005	....
5	0.1000	0.0701	....	70.1
6	0.1000	0.0700	....	70.0
7	0.1000	0.0644	....	64.4
8	0.1000	0.0644	....	64.4
9	0.2000	0.0812	....	40.6
10	0.2000	0.0808	....	40.4
11	1.0000	0.0107	....	1.07
12	1.0000	0.0110	....	1.10
13	1.0000	0.0231	....	2.31
14	1.0000	0.0243	....	2.43
15	1.0000	0.0492	....	4.92
16	2.0000	0.0998	....	4.99
17	1.0000	0.0055	....	0.55
18	1.0000	0.0099	....	0.49
19	0.2000	0.1134	....	56.7[a]

[a] Average of 56.4, 56.8, and 57.0.

residue settles completely. Filter through a 9-cm. No. 40 Whatman paper, wash the residue with 1 to 1 nitric acid, then transfer it to the filter with hot water. Place the filter in the beaker, cover it with a little concentrated sulfuric acid, and fume with small additions of sodium nitrate until oxidation of the paper is complete. Add a little water and transfer the solution to a large platinum crucible for evaporation with hydrofluoric acid to fumes of sulfur trioxide. Dissolve the sulfates of tin, antimony, or possibly lead in 20 ml. of 1 to 4 hydrochloric acid, transfer the solution to a 100-ml. volumetric flask, and make up to volume. The oxides of antimony and tin should not be ignited because they form a solid mixture.

Transfer 50 ml. of the solution to the special flask for reduction with 4 to 5 grams of zinc, using the mossy variety for slow action. Allow the reduction to proceed for half an hour with zinc still in excess, then add 25 ml. of hydrochloric acid and heat to boiling for about 5 minutes. Cool quickly with the flask closed, release the vacuum, and filter off antimony and lead on a fast paper, such as S. & S. 597, directly into 15 ml. of mercuric chloride solution with stirring. Wash once with dilute hydrochloric acid. A small precipitate of mercurous chloride settles slowly. Weigh the mercurous chloride and calculate to tin as described above.

Nos. 17 and 18, Table I, are duplicates on a sample high in sulfides. No. 18 contained 5.0 mg. of standard tin purposely added in excess of the quantity in the ore.

Summary

The chief advantages of this method are the use of a small volume of stannous chloride for the precipitation of mercurous chloride and the simplicity of reducing the tin. Lead and antimony in minor quantities are the only metals likely to interfere, but their relative insolubility in dilute acid after reduction with zinc makes the interference negligible for most ores. This method is more easily controlled than the iodometric, and will handle most types of ore, according to the indicated procedure. Concentrates of 70 per cent tin, or more, are completely reduced by a single fusion with sodium cyanide. Much time is saved by one short reduction.

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Determination of Small Amounts of Arsenic, Antimony, and Tin in Lead and Lead Alloys

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A new method for the determination of small amounts of arsenic, antimony, and tin in lead and lead alloys consists of separation of the three metals from the lead by a double coprecipitation with manganese dioxide, reduction of arsenic and antimony to the trivalent state, separation of the arsenic by distillation as chloride, titration of the arsenic and antimony separately by the method of Györy, and reduction of tin with lead and titration with standard iodine solution.

Methods for the determination of small amounts—i. e., 0.001 to 0.02 per cent—of arsenic, antimony, and tin in lead and lead alloys usually call for the preliminary removal of lead as sulfate. This separation is not satisfactory, however, unless hydrofluoric acid is added to ensure complete solution of the three metals (*17*). If hydrofluoric acid is used, platinum or ceresin vessels are required unless the glassware is known to be arsenic-free. Recently a very accurate method of separating lead from its impurities has been described (*4*), but it requires special apparatus and is not suitable for rapid analysis.

Several years ago a method was developed in these laboratories which has proved to be very satisfactory for routine analysis of lead alloys (see Table II). In this method the arsenic, antimony, and tin are separated as hydrated oxides from the nitric acid solution of the alloy by coprecipitation with hydrated manganese dioxide (*2*, *11*). After separation from the lead, the metal oxides are converted to sulfates, and arsenic and antimony are reduced to the trivalent state. Arsenic is then separated by distillation as chloride, and titrated with standard bromate solution by the method of Györy (*8*). The solution containing the tin and antimony is diluted with hot water, and the antimony is titrated by the Györy method. Finally, tin is reduced with lead and titrated with standard iodine solution (*9*).

The method is applicable to the analysis of pig lead and all the usual types of lead alloys except those containing selenium, tellurium, and large amounts of antimony and tin. Methods of analysis employing coprecipitation separations have been developed for the latter alloys, but will not be considered here.

Apparatus

The apparatus required for the distillation of arsenic is shown in Figure 1. It consists of a 500-ml. Erlenmeyer flask with a three-hole rubber stopper carrying a 0.6-cm. (0.25-inch) glass tube, a thermometer, and a glass capillary pressure regulator

which comes within 0.3 to 0.6 cm. (0.125 to 0.25 inch) of the bottom of the flask. The inside diameter of the capillary is 2 mm.

Reagents

Standard Potassium Bromate Solution (0.01 *N*). Recrystallize c. p. potassium bromate twice from water and dry to constant weight at 180° C. Dissolve 0.2783 gram in water and dilute to 1 liter in a volumetric flask.

Standard Tin Solution. Dissolve 1.000 gram of c. p. tin in 500 ml. of hydrochloric acid with the aid of a few crystals of potassium chlorate. Transfer to a 1-liter volumetric flask and dilute to 900 ml. Cool to room temperature and dilute to the mark.

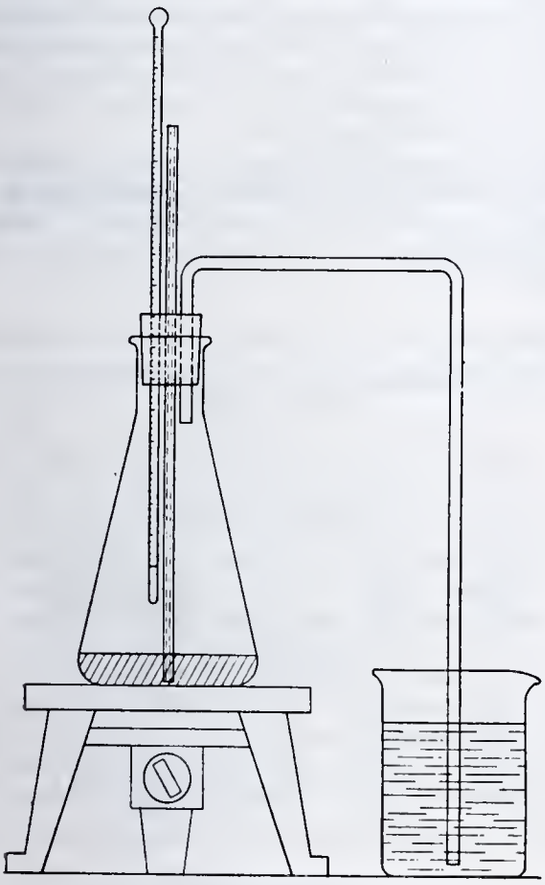

Figure 1

Standard Iodine Solution (0.01 *N*). Dissolve 1.2 grams of c. p. iodine in 35 ml. of water containing 20 grams of potassium iodide, and dilute to 1 liter.

To standardize this solution, pipet 10 ml. of standard tin solution (1 gram of tin per liter) into a 500-ml. wide-mouthed Erlenmeyer flask, add 10 ml. of sulfuric acid, 75 ml. of hydrochloric acid, and 20 grams of sodium chloride, and dilute to 350 ml. with water. Add 5 grams of coarse-grain test lead and reduce and titrate with the iodine solution as directed in the procedure.

Procedure

Weigh 50 grams of the milled sample into a 500-ml. Erlenmeyer flask, add 150 ml. of water and 50 ml. of nitric acid, and heat gently to dissolve the sample. Dilute to 300 ml. and heat to boiling to expel brown fumes. Add 10 ml. of potassium permanganate (2 per cent solution) to oxidize the arsenic, antimony, and tin. Add 20 ml. of manganese nitrate (10 per cent solution), and boil gently for 2 minutes. (The manganese nitrate must not be added before the potassium permanganate.) Filter hot through a rapid paper and wash well with hot water to remove as much lead nitrate as possible. Reserve the filtrate. Return the paper to the 500-ml. Erlenmeyer flask, add 15 ml. of sulfuric acid and 35 ml. of nitric acid, and heat to gentle boiling to destroy the filter paper.

Add 35 grams of pure ammonium sulfate, dissolved in 100 ml. of water, to the reserved filtrate and cool to room temperature. Filter through close paper on a Büchner funnel, refiltering if the solution is not clear. Wash once or twice with water. Discard the lead sulfate. Neutralize with ammonium hydroxide to the blue color of copper ammonium ion, or to litmus if the lead sample does not contain copper. Add 15 ml. of ammonium hydroxide in excess, heat to boiling, add 10 ml. of ammonium persulfate (10 per cent solution), and boil vigorously for 1 minute. Filter through a rapid paper. Wash the precipitate onto the paper. (Ignore the small amount of precipitate which cannot be washed from the walls of the beaker.) Wash the precipitate 3 or 4 times with hot water and discard the filtrate. Place the paper in the 500-ml. flask with the first paper. The flask now contains all the arsenic, antimony, and tin, plus some manganese, lead, and traces of other elements such as iron and copper.

Add 35 ml. of nitric acid and boil gently to destroy all carbonaceous material. Take to copious white fumes to expel nitric acid and to dissolve all lead sulfate. (Incomplete solution of the lead sulfate indicates that the manganese dioxide precipitates have not been adequately washed. Sufficient sulfuric acid to ensure complete solution must be added before proceeding.) Cool, add 100 mg. of hydrazine sulfate and 3 grams of potassium acid sulfate, and wash down the flask with about 10 ml. of water, making sure that no hydrazine sulfate remains on the walls. Boil to expel the water. Take to copious white fumes over a flame and fume strongly for 30 to 60 seconds to ensure complete expulsion of sulfur dioxide.

Cool and add 10 ml. of water. Cool to 25° C., add 50 ml. of hydrochloric acid, and a few grains of 12-mesh silicon carbide to prevent bumping. Stopper with a distillation head (see Figure 1), and heat to boiling on a hot plate, catching the distillate in 300 ml. of water in a 400-ml. beaker. (It is not necessary to keep this water cold, since arsenic trichloride cannot be distilled from solutions containing less than 16 per cent of hydrochloric acid, *20*.) Boil the solution at a moderate rate until the temperature of the vapor rises to 105° C. When this temperature is attained remove the flask from the plate and detach the distillation head. Dilute the solution containing the tin and antimony to 275-ml. volume. Heat to gentle boiling to dissolve all lead salts. (Failure to dissolve lead salts will lead to low results for antimony. If complete solution is not attained, add 10 grams of sodium chloride and boil to dissolve all salts.) Titrate this solution, and that containing the arsenic, at 95° C. with standard potassium bromate, using methyl orange as indicator. Provide for blank correction.

$$\text{Ml. of } KBrO_3 \times 0.001218 = \text{per cent antimony}$$
$$\text{Ml. of } KBrO_3 \times 0.0007493 = \text{per cent arsenic}$$

Add 50 ml. of hydrochloric acid and 5 grams of coarse-grain test lead to the solution, after the antimony titration, and boil at a moderate rate for about 15 minutes. [If the original sample contains copper and less than 2 mg. of antimony add 5 mg. of antimony (as antimony trichloride) with the hydrochloric acid.] When deposition of the noble metals is complete, filter through cotton or glass wool into a 500-ml. wide-mouthed Erlenmeyer flask containing 5 grams of test lead and 20 grams of sodium chloride. Wash with warm water. Cap with a tin reduction air condenser (*9*) and start a slow flow of carbon dioxide. Heat to boiling and boil at a moderate rate for 45 minutes. Increase the flow of carbon dioxide and remove the flask to an ice bath. Cool to 5° to 10° C. Add 5 ml. of potassium iodide (10 per cent solution), 5 ml. of starch (1 per cent solution), and titrate, under a blanket of carbon dioxide, with standard iodine solution 0.01 *N*. Provide for blank correction.

$$\text{Ml. of iodine} \times \text{tin factor} \times 2 = \text{per cent tin}$$

Discussion

Separation of arsenic, antimony, and tin from nitric acid solution of a lead alloy by coprecipitation with manganese dioxide is not complete with a single precipitation. The separation of tin is about 99 per cent complete and that of antimony and arsenic about 95 and 90 per cent, respectively. By employing a double precipitation from acid solution, close to 100 per cent of the tin can be recovered, but the antimony and arsenic recovery is not much improved. Neutralization of the solution, as recommended by Fainberg (*6*), greatly improves the separation of arsenic, but does not appear to help the antimony. Moreover, neutralization of the solution results in heavy contamination of the precipitate with lead and copper salts. The only satisfactory method found was to make a second coprecipitation from ammoniacal solution after preliminary removal of the lead as sulfate.

Table I. Separation of Arsenic with Hypophosphorous Acid

No.	Arsenic Present	Arsenic Found	Error
	Mg.	*Mg.*	*Mg.*
1	0.5	0.5	0.0
2	1.0	1.0	0.0
3	1.0	0.9	−0.1
4	1.0	0.9	−0.1
5	2.5	2.4	−0.1
6	2.5	2.4	−0.1
7	5.0	4.9	−0.1
8	5.0	4.9	−0.1
9	10.0	9.8	−0.2
10	10.0	9.9	−0.1

Reduction of arsenic and antimony in concentrated sulfuric acid solution, previous to distillation or volumetric determination, is best accomplished with the aid of hydrazine sulfate (*18*). After reduction, the fuming to remove sulfur dioxide must be very vigorous, but not unduly prolonged if copper is present, because oxidation of antimony and especially of arsenic by the oxygen in the air is greatly accelerated by copper ions (*21*). Traces of iron and copper accompanying the arsenic, antimony, and tin are reduced by the hydrazine sulfate, but are reoxidized during the fuming to expel sulfur dioxide, and hence are not harmful in subsequent Györy bromate titrations.

One of the methods often used for obtaining antimony and arsenic in the trivalent state is to reduce with sulfite or sulfur dioxide. It appears, however, that the limitations of this method, so thoroughly elucidated by Kurtenacker and Fürstenau (*14*), are not always understood (*1*).

Previous to the development of the distillation method used in the procedure, two other methods for the determination of arsenic and antimony were tested. In the first method the two metals were reduced to the trivalent state with hydrazine sulfate and titrated with bromate. Arsenic was then separated with hypophosphorous acid (*5*), dissolved in hot sulfuric acid, and again titrated with bromate. Antimony was determined by difference. This method was fairly satisfactory but the results for arsenic were always slightly low and those for antimony correspondingly high.

It has been the author's experience that the hypophosphorous acid method invariably yields low results for arsenic (see Table I). Mercury (*12, 16*), copper ion (*5*), and iodide (*14*) act as catalysts in the precipitation of the arsenic but do not improve the results.

Many methods have been proposed for the solution and titration of arsenic after precipitation with hypophosphorous acid (*1, 7, 13, 19*). Perhaps the most convenient is that of Anderson (*1*) in which the arsenic is dissolved in hot concentrated sulfuric acid and titrated, after dilution, by the method of Györy. (Apparently Anderson overlooked the fact that hydrochloric acid must be present in the Györy titration, and that reduction with sulfite is ineffective and unnecessary.)

The second method tested is similar to the first, except that after the initial bromate titration the antimony is selectively reduced with mercury as recommended by McCay (*15*) and again titrated with bromate. Arsenic is determined by difference. (Tin in the solution is then reduced with lead and titrated with standard iodine.) The method yields very satisfactory results in the analysis of arsenic, antimony, and tin in lead alloys (see Table II).

The reduction of antimony in the presence of arsenic by shaking with mercury is satisfactory, providing calomel is not formed. With a hydrochloric acid concentration of at least 30 per cent, the mercury dissolves to form a soluble mercuric chloride complex and the reduction is complete for small amounts—i. e., less than 5 mg.—of antimony with a 5-minute shaking. The reduction of larger amounts of antimony is not quite complete. Experiments show, however, that the addition of a few milligrams of copper as catalyst results in complete reduction with a 5-minute shaking.

In the procedure, lead is used to reduce tin previous to titration. Very few of the metals usually found in lead alloys interfere. Some tin is lost by codeposition with antimony (*3, 10*), but the loss is negligible even in the presence of as much as 0.5 gram of antimony. Loss of tin by codeposition with copper is much more serious (*3*). The presence of as little as 2 mg. of copper will cause the results to be appreciably low. Fortunately, however, the deleterious effect of copper is greatly reduced if antimony is present (or is added) before reduction of the tin with lead. Apparently, when antimony is present, the copper tends to deposit on the lead in the form of a copper-antimony alloy rather than a copper-tin alloy.

One disadvantage of the use of lead as reductant is that lead chloride may precipitate as the solution is cooled before titration. Deposition of noble metals on the lead greatly accelerates solution of the latter in hydrochloric acid. For this reason it is best to replace the contaminated lead with fresh lead as soon as the noble metals have all precipitated.

In the lead reduction method it is not necessary to remove the lead from the solution before titration, providing sufficient excess of iodide is present to ensure a stable end point. When determining small amounts of tin, iodide must be added before the titration.

Table II. Determination of Arsenic, Antimony, and Tin in Lead Alloys

No.	Arsenic Present	Arsenic Found	Antimony Present	Antimony Found	Tin Present	Tin Found
	Mg.	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*
			Indirect Method			
1	1.0	1.1	6.6	6.6	6.5	6.4
2	1.0	1.0	2.0	2.0	1.7	1.6
3	5.0	5.0	5.3	5.3	5.3	5.2
4	5.0	5.0	5.5	5.6	5.5	5.5
5	10.0	9.9	1.0	0.9	10.6	10.5
6	10.0	10.0	10.5	10.6	1.7	1.6
			Direct Method			
1	1.7	1.7	1.5	1.6	1.4	1.4
2	2.7	2.6	3.1	3.1	2.4	2.4
3	2.9	2.9	1.7	1.7	4.1	4.0
4	4.2	4.2	5.1	5.0	6.6	6.7
5	5.7	5.6	4.7	4.6	3.4	3.3
6	10.7	10.6	8.4	8.4	9.1	9.0
7	11.3	11.2	9.6	9.5	11.2	11.2

Experimental

Aliquot portions of a standard arsenite solution were diluted with water to 50 ml. in a 250-ml. beaker, 50 ml. of hydrochloric acid and 10 ml. of hypophosphorous acid were added, and the arsenic was separated and determined by the modified Anderson method. The results are shown in Table I.

Weighed quantities of arsenious oxide, antimony, and tin were boiled gently with 10 ml. of nitric acid; 80 grams of lead nitrate and 100 mg. of cupric nitrate hexahydrate were added; the sample was diluted to 300 ml.; heated to boiling to dissolve all soluble salts; and arsenic, antimony, and tin were then separated and determined as directed in the procedure, except that the antimony was selectively reduced with mercury by McCay's method and the arsenic was determined by difference. The results are shown in Table II (indirect method).

Weighed quantities of arsenious oxide, antimony, and tin were boiled gently with 50 ml. of nitric acid until dissolution was complete; 250 ml. of water and 50 grams of c. p. milled lead were added; the lead was dissolved by gentle heating and the arsenic, antimony, and tin were then separated and determined as directed in the procedure. The results obtained are recorded in Table II (direct method).

Acknowledgment

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Fixing and Determining Oil in Feed Water and Boiler Water

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The two sources of error encountered in the determination of oil in water samples are: (1) the presence in the oil residue after evaporation of substances other than oil, such as the inorganic salts, sodium sulfate, and sodium chloride. These substances increase the apparent oil content of the water sample and produce high results. (2) The separation of oil from the water sample on standing. This oil may separate onto the sides of the container, from which it may be incompletely removed even by rinsing the container with a solvent. This loss of oil from the water sample itself yields a lower apparent oil content and gives low results.

Data are presented on the magnitude of the error involved, using present methods.

A new procedure is recommended for fixing the sample at its source by adsorbing oil in a ferric hydroxide precipitate and subsequently extracting the oil from the washed and dried floc. It is possible by this method to avoid both of the above sources of error and to obtain consistent, accurate results.

OIL is both undesirable and dangerous in a boiler water, particularly under modern operating conditions. The presence of even a very small quantity of oil may lead to foaming and priming of the boiler water, corrosion of the boiler metal, and overheating of boiler tubes, resulting in possible failure. The overheating of the tubes may be due either to the heat-insulating properties of a layer of oil or to the effects of oil on scale formation. Oil absorbed by suspended solids in the boiler water may adversely affect the ability of such solids to remain in suspension. Oil is considered such an important factor in the production of pure steam that the American Boiler Manufacturers' Association, in its guarantees of steam purity, stipulates an oil content of the concentrated boiler water not exceeding 7 p. p. m.

Methods for the removal of oil from boiler feed water or returned condensate range from mechanical baffle-type separators to complete chemical coagulation and filtration systems. Since a guarantee on the purity of steam from a given boiler may definitely hinge on the oil content of that boiler water, oil determinations of the boiler water must be made as accurately as possible. There are admitted difficulties in securing a representative sample of a boiler water for oil, as the oil may partially be found in the metal or absorbed by solid matter, such as sludge. In many cases (given as an alternate by the A. B. M. A. specifications) the practice has been to secure a sample of the boiler feed water and first concentrate it at low temperature and pressure to the same number of cycles of concentration as the boiler water before making the oil analysis. Inasmuch as 5.0 per cent of blowdown, equivalent to 20 cycles of concentration, may be considered an average case, if the boiler water is not to exceed 7 p. p. m. of oil, the feed water should contain no more than 0.35 p. p. m. of oil.

Water samples lose their oil content on standing as the oil separates from the water and adheres to the sides of the container or stopper. Such oil may be incompletely removed, even by rinsing the container with a solvent. Concentrating the sample prior to analysis is undesirable, owing to the separation of the oil which may take place during concentration; on the other hand, analysis of the unconcentrated feed water requires a minimum of errors, because of the small quantities involved. A truly comprehensive study of boiler feed water for oil would require spot samples, taken at sufficiently frequent intervals to detect suspected variations in oil content. Obviously, a continuous sample will be subject to some oil separation, owing to the time required for collection, before the sample can be fixed or analyzed.

An investigation by the authors has shown errors in the methods for oil determination now available. The following methods have been considered: (*a*) standard method (*2*), (*b*) technical method (*3*), (*c*) ether extraction method, (*d*) A. B. M. A. method (*1*), and (*e*) Scott's method (*5*).

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Table I. Comparison of Methods on Oil-Distilled Water Solution

Sample	Oil Values: Ether-extraction method	Technical method	Authors' procedure
	P. p. m.	*P. p. m.*	*P. p. m.*
1	0.0	0.0	0.0
2	4.4	4.8	5.6
3	11.4	12.4	12.4
4	22.6	21.0	21.5
5	...	42.6	42.2
6	380.0	374.8	362.2

The standard method (*2*) is described as a rough, quantitative method in which 2 to 4 liters of the water are evaporated to dryness at a temperature below 100° C. The residue is taken up with petroleum ether, or high-test (oil-free) gasoline in a tared, platinum dish, or small light beaker, and evaporated to dryness on a steam bath. This reference cites "the technical method" (*3*) as more precise.

The technical method (*3*) involves transferring a water sample to a separatory funnel and shaking with redistilled chloroform. The water and chloroform layers are separated and the latter is evaporated in a weighed flask and dried to constant weight at 100° C. This method is subject to an error, due to the fact that some of the dissolved salts in the water, such as sodium chloride, sodium sulfate, etc., are taken up by the chloroform and finally weighed as oil.

The ether-extraction method is the same as the technical method, except that ether is used instead of chloroform, and is subject to the same error.

The A. B. M. A. standard contract (*1*) states, "The total quantity of oil, or grease, or substances which are extractable either by sulfuric ether or by chloroform, shall not exceed 7 p. p. m. in the boiler water when the sample being tested is acidified to 1 per cent hydrochloric acid, or 7 p. p. m. in the feed water when the sample being tested is first concentrated at low temperature and pressure to the same p. p. m. total solids as the boiler water." The hydrochloric acid is used to remove from the water solution any saponifiable oil. This method also yields high results due to the soluble salt taken up by the chloroform.

Scott's method (*5*) uses ferric hydroxide to absorb the oil from a sample and subsequently extracts the oil. This method is essentially the same as that used by the authors, except in the matter of fixing the sample at its source. Its major disadvantage is that oil which has separated from the sample on standing is not absorbed.

Inaccuracies involved in all the above mentioned methods may be from one or both the following sources of error:

1. The presence in the oil residue after evaporation, of substances other than oil, such as the inorganic salts sodium sulfate and sodium chloride. These substances increase the apparent oil content of the water sample and produce high results.
2. The separation of oil from the water sample on standing. This oil may separate onto the sides of the container, from which it may be incompletely removed, even by rinsing the container with a solvent. This loss of oil from the actual water sample itself yields a lower apparent oil content and gives low results.

This paper presents a method of fixing the sample at its source, so that an accurate result may be obtained later in the laboratory by a method similar to Scott's (*5*). Data are presented to show the errors inherent in the other methods.

Preparation of Oil Standards

Paraffin oil was selected for these studies because of its inertness and low volatility. The latter is important, particularly in view of the fact that the oil samples after their extraction from water are placed in an oven to dry. The loss in weight, due to the volatility of the paraffin oil employed, was 0.012 per cent after drying at 105° C. for 15 minutes and 0.26 per cent after drying for 30 minutes. Oils normally encountered in water have low volatility.

Considerable difficulty was experienced in preparing a standard water-oil solution. Oil separated rapidly from the water in a simple mixture of oil with distilled water, even with the most vigorous stirring. Unlike Pringle (*4*), the author did not find it possible to add a definite weight of oil to a water sample and to check the addition of weight on aliquot samples. The method finally adopted was to mix a small amount of paraffin oil intimately with distilled water, employing a laboratory hand homogenizer and then thoroughly mixing this solution with a larger volume of distilled water. This latter solution exhibited a milky appearance, similar to the appearance of samples drawn from oil-contaminated return condensate systems. Even with this homogenization, however, small oil droplets separated from the body of the solution and gradually floated to the top. Changes in the concentration of oil in the mixture from time to time necessitated siphoning of the sample from beneath the surface and the simultaneous securing of the duplicate samples for the test by the different methods. In each case, 500 ml. of the water-oil solution were taken. Even using this procedure it was not possible to check the amount of oil originally added to the water sample, although it was possible to duplicate oil values on samples simultaneously siphoned off and immediately analyzed. In an attempt to check the oil content of the oil-water solution, portions were siphoned and evaporated to dryness. Separation of oil on the sides of the tared container during evaporation exposed this oil to prolonged heating and resulted in volatilization of the oil and erratic results.

Table II. Solvent Effect of Different Methods on Oil-Free Samples

Solutions	Apparent Oil Content, P. P. M.: 1000 p. p. m. NaCl	5000 p. p. m. NaCl	1000 p. p. m. Na_2SO_4	5000 p. p. m. Na_2SO_4	1000 p. p. m. Na_2CO_3	5000 p. p. m. Na_2CO_3	1000 p. p. m. NaOH	5000 p. p. m. NaOH	100 p. p. m. tannic acid	200 p. p. m. tannic acid
				Ether Extraction Method						
50 ml. of ether	0.8	5.6	0.6	3.6	0.8	5.4	0.6	5.2	1.0	0.8
100 ml. of ether	2.0	7.6	2.2	7.0	2.2	9.4	3.0	11.2	1.2	2.2
150 ml. of ether	2.0	11.6	2.2	10.2	4.0	14.6	3.2	15.8	1.4	3.0
				Technical Method						
50 ml. of chloroform	0.2	0.6	0.0	1.0	0.2	1.8	0.8	1.0	0.6	[a]
100 ml. of chloroform	0.4	0.8	0.2	1.2	0.4	3.0	1.0	1.6	1.2	[a]
150 ml. of chloroform	0.6	1.0	0.4	2.0	0.6	3.4	1.4	1.8	1.0	[a]
				Authors' Method						
1-hour reflux	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2-hour reflux	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
				A. B. M. A. Method						
50 ml. of ether	0.6	4.0	0.6	5.8	1.4	3.8	1.0	1.5	2.0	2.4
100 ml. of ether	2.8	10.0	2.8	15.8	6.6	10.6	4.8	16.2	5.4	6.2
150 ml. of ether	2.9	10.0	4.4	15.8	7.0	14.6	7.6	19.8	10.6	11.4

[a] Too much foam developed for good separation.

Authors' Procedure

Preparation of Ferric Chloride Solution. Ten grams of ether-washed iron by hydrogen are placed in a 600-ml. beaker to which are added 400 ml. of distilled water, followed by 30 ml. of concentrated c. p. hydrochloric acid. After solution is complete, c. p. concentrated nitric acid is added until the ferrous chloride is completely oxidized and a deep, amber color per-

sists. This solution is stirred constantly during the addition of the nitric acid. It is boiled for approximately 0.5 hour, cooled, and made up in volume to 1 liter with distilled water.

Preparation of Ammonium Hydroxide Solution. Concentrated ammonium hydroxide (360 ml., specific gravity 0.9) is made up to 1.0 liter with distilled water.

Ether. Anhydrous ethyl ether (fat-free).

Procedure for Test. Five hundred milliliters of the sample are treated with 10 ml. of the ferric chloride solution, and stirred well, and then 10 ml. of the ammonium hydroxide solution are added and stirred. The solution, now fixed, is brought to a boil for approximately 1 minute, cooled to room temperature, and filtered by gentle suction through an Alundum thimble, which has previously been ignited. The thimble containing the ferric hydroxide is washed free from chloride with distilled water, dried at 105° C. for 1.5 hours, and cooled to room temperature, and the oil is extracted in a Soxhlet apparatus in the usual manner for 1 hour. It was found necessary to use an Alundum thimble instead of filter paper, because of ether- and chloroform-extractable matter in the filter paper.

Table III. Solvent Effect of Different Methods on Oil-Water Samples Containing Sodium Chloride

Method	Apparent Oil Content, P. P. M. Sample 1, 1000 p. p. m. NaCl	Sample 2, 2000 p. p. m. NaCl	Sample 3, 3000 p. p. m. NaCl	Sample 4, 5000 p. p. m. NaCl
Ether extraction	19.2	20.0	47.2	77.0
Technical	18.4	20.1	46.2	73.0
Authors'	18.0	19.5	45.6	70.2

Experimental Data

Table I illustrates the values secured by three different methods for determining the oil content of oil–distilled water solutions, and indicates that in distilled water solutions the technical method and the authors' method compare very favorably. There is no essential difference between these methods on distilled water solutions free from dissolved salts and on samples simultaneously obtained and immediately analyzed. Using the authors' method, the dried ferric hydroxide containing no oil gave no measurable increase in weight after being refluxed for 2 hours with ether.

In order to illustrate the increase in weight and apparent oil content found by the various methods, due to the presence of different salts dissolved in the water sample, solutions containing 1000 and 5000 p. p. m. of sodium chloride, sodium sulfate, sodium carbonate, and sodium hydroxide and solutions containing 100 and 200 p. p. m. of c. p. tannic acid were prepared with oil-free distilled water. Oil determinations were made on each sample in accordance with the procedures described.

Table II illustrates the apparent oil content of these solutions. With the ether-extraction method, particularly with the sample containing 5000 p. p. m. of these salts, the apparent oil content increased with the use of increasing quantities of ether employed in the extraction. This is apparent to a lesser extent with the solutions of lower concentration.

With the technical method, the apparent oil content also increased with increase in the quantity of chloroform employed in the extraction. With both of these methods, and to a greater extent with the A. B. M. A. method, this increase in weight with greater volume of solvent can be ascribed to the increased quantity of water dissolved in the solvent, with accompanying increased quantity of dissolved salt. The authors' method illustrates that it is possible to avoid the error due to the presence of dissolved salts in the solvent residue after evaporation, inasmuch as the ferric hydroxide precipitate containing the oil is washed free from solids by distilled water before it comes in contact with the solvent in the Soxhlet apparatus.

To determine the solvent effect when oil is actually present in water samples, sodium chloride was used in concentrations of 1000, 2000, 3000, and 5000 p. p. m. These solutions were made in homogenized oil–distilled water samples, collected, and measured in the manner previously described. The results are illustrated by Table III.

The lowest oil values were consistently shown with the authors' procedure, and the ether extraction and technical methods were consistently higher in apparent oil content. This is due to the residue of sodium chloride remaining on evaporation of the solvent, as illustrated by Table II.

The increase in apparent weight of oil with the ether extraction and technical methods, compared with the authors' method, closely checks the solubility of sodium chloride in these solvents shown by the first column of Table II with the use of the same volume of solvent. For all the results shown by Table III, 50 ml. of chloroform were used with the technical method and 100 ml. of ether with the ether extraction method. While the results obtained by the three methods do not vary to a great extent, these values were obtained on freshly prepared oil samples which were immediately analyzed. In order to illustrate the loss of oil resulting from the separation of oil from the water on standing, samples of oil-contaminated boiler feed water from nearby industrial plants were obtained through a cooling coil and collected in a 5-gallon bottle. The individual samples were siphoned into 500-ml. volumetric flasks and placed in Pyrex bottles. The samples which were to be determined with the use of the authors' procedure were all fixed at the time of sampling.

Results shown in Table IV are for oil determinations made immediately after collection and then at intervals of 2, 4, and 6 days. A definite decrease in the oil content of the unfixed samples can be noted after 2 and 4 days, when the analysis was made by the ether extraction or technical method. In each case the bottle was rinsed with a portion of the solvent to attempt to collect oil clinging to the sides of the container. Employing the authors' procedure for fixing and determining the oil content, no drop in oil content was noted. Drop in oil content, because of oil separating from solution and clinging to sides of container, is apparently prevented by the adsorption of the oil in the ferric hydroxide precipitate using the authors' procedure.

Table IV shows clearly that it is necessary to fix the samples for oil determination in order to secure an accurate result. The low solids content of these feed-water samples did not affect the final result to any great extent. The residue left after the oil contamination gave a slight test for sulfate and chloride on the samples analyzed by the ether extraction and technical methods. No sulfate or chloride was found in the residue using the authors' procedure.

Table IV. Separation of Oil from Water Samples on Standing

Sample	Oil Content, P. P. M. Immediately after collection	Two days after collection	Four days after collection	Six days after collection
Ether Extraction Method				
A	2.4	1.0	0.8	..
B	10.4	8.8	5.9	4.8
Technical Method				
A	2.3	0.8	0.5	..
B	9.9	8.5	5.5	4.4
Authors' Method				
A	2.2	2.3	2.2	..
B	10.0	10.1	9.9	10.2

Mineral Analyses of Feed Water[a]

	Sample A	Sample B
Total hardness as $CaCO_3$	16	6
Sulfate as SO_4	4	48
Chloride as Cl	1	3.5
Iron as Fe	0.0	Trace
P alkalinity as $CaCO_3$	0	0
M alkalinity as $CaCO_3$	22	16
Total solids	45	108
pH	7.1	7.1

[a] Values in p. p. m. with exception of pH.

In order to show the solvent effect on actual boiler water, boiler waters having different oil contents were secured from industrial plants and the oil was determined immediately after their collection (Table V). The consistent check values obtained by means of the authors' procedure compare very favorably with those previously secured from synthetic oil-water samples. Close checks were obtained in each case with the use of the authors' procedure, while the other three methods showed wide variation on check samples. The residues were tested in each case for the presence of sulfate and chloride. Sulfate and chloride were present except in samples determined by the authors' procedure. In the case of the A. B. M. A. method, the residue also showed the presence of iron. The consistent checks obtained with the use of the authors' method and the absence of chlorides, sulfate, etc., in the residue illustrate the importance of fixing a sample for oil prior to its determination in order to avoid erratic results.

Table V. Comparison of Methods on Boiler Water Samples

	Oil Content, P. P. M.				
	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Ether-extraction method	33.5	33.5	58.0	374.0	62.5
	28.5	20.1	56.7	356.0	58.6
Technical method	43.3	23.3	55.0	365.0	55.1
	28.7	22.0	42.5	450.0	49.3
A. B. M. A. method	52.0	20.5	95.5	328.0	71.0
	48.8	24.0	72.4	..	33.0
Authors' procedure	26.2	18.2	47.0	382.2	57.2
	26.0	18.2	47.4	383.0	56.8
Mineral Analyses of Boiler Waters[a]					
Total hardness as $CaCO_3$	0	0	0	26	100
Sulfate as SO_4	260	272	304	352	144
Chloride as Cl	168	100	44	60	168
Iron as Fe	0.0	0.0	0.0	0.0	0.0
P alkalinity as $CaCO_3$	154	102	124	124	120
M alkalinity as $CaCO_3$	270	310	264	344	400
Total solids	1730	1786	1408	3336	4321
pH	10.2	10.2	10.1	10.1	10.1
Phosphate as PO_4	25	30	25	..	..

[a] Values in p. p. m. with exception of pH.

Recommended Procedure for Fixing and Determining Oil Content of Water Samples

Collecting the Sample. Wash a 500-ml. volumetric flask with a small amount of 95 per cent alcohol, rinse out the alcohol with a small amount of ethyl ether, and dry the inside of the flask with a stream of air. Wash a 500-ml. bottle and cork stopper in the same fashion.

Measure 500 ml. of the water sample into the volumetric flask at the sampling point. Pour the contents of the flask into the bottle and rinse the flask with a small amount of oil-free distilled water which is also added to the 500-ml. bott e. By means of a graduate, add 10 ml. of the ferric chloride solution, stopper, and shake well. Then add 10 ml. of the ammonium hydroxide solution, stopper, and shake well. The sample is now "fixed".

Determination. Set up a filter flask and insert an Alundum thimble in the filter tube, fastening by means of a wide rubber band. The Alundum thimble should first be ignited in a Bunsen flame for approximately 5 minutes.

Add the fixed sample to a 1-liter beaker (ether-washed) and rinse the bottle well with oil-free distilled water, bring the sample just to a boil on a hot plate, and let stand until cool (below 40° C.). Filter the fixed sample through the Alundum thimble, using a gentle suction. Wash the beaker well, making certain that all of the ferric hydroxide floc is washed into the thimble. Then wash the thimble with distilled water, testing the filtrate from time to time with 0.1 N silver nitrate solution until no further opalescence appears. Let the thimble dry with gentle suction and then place the thimble in an oven at a temperature of 105 ± 1° C. for 1.5 hours. Remove and let cool in a desiccator.

Weigh a Soxhlet flask which has been previously ether-washed and dried at 100° C. for one hour and cooled in a desiccator, and add approximately 75 ml. of ethyl ether. Extract the cooled thimble and floc in the Soxhlet apparatus for one hour on a steam bath. Evaporate the ether. Dry the outside of the flask, place in an oven at 100° C. for 15 minutes, cool, and weigh. The increased weight of the Soxhlet flask in milligrams, multiplied by 2, equals p. p. m. of oil.

Discussion and Conclusions

One source of error involved in the various methods for determining oil in water is the presence of inorganic salts in the oil residue when weighed. In certain instances, this error may be appreciable. The error due to inorganic salts is most noticeable on water samples containing high dissolved solids content, such as boiler water. Since the specification limit on oil in a boiler water is only 7 p. p. m., it is necessary to employ a method of oil analysis that will not register erroneously high simply because of inorganic salts such as sodium sulfate and sodium chloride in the oil residue.

A second and more important source of error is due to the loss of oil from the water sample on standing. It is seldom convenient to run oil samples immediately at their source. The authors' method of "fixing" the sample, immediately upon collection satisfactorily maintains the original oil content even after prolonged standing.

The difficulty in preparing standard oil-water solutions made it necessary to prove the accuracy of the proposed method by indirect means. Had it been possible to add a definite amount of oil to a water sample and then to check this addition by determination of the oil on aliquot portions, the percentage adsorption of oil could have been proved by the ferric hydroxide precipitate. No method employed, even direct evaporation of an oil-water sample, checked the initial addition of oil to a water sample. However, on oil–distilled water samples, freshly prepared and immediately analyzed, check results were obtained by the technical method, ether extraction method, and the authors' procedure, indicating complete adsorption of oil by the ferric hydroxide precipitate within the limits of error of these tests.

The authors' procedure does not determine saponifiable oil. However, lubricating oil as usually employed throughout the power plant cycle contains only a small percentage of saponifiable oil. While the method recommended by the American Boiler Manufacturers' Association, acidification to 1 per cent hydrochloric acid, can determine the saponifiable oil, this slight advantage is overweighed by the increase in solids content due to the presence of inorganic salts and to the change in oil content from time of sampling to time of analysis.

This recommended procedure has been employed in the laboratories of W. H. & L. D. Betz for the past four years, during which time between four and five thousand determinations of the oil content of water samples have been made. The method has been found satisfactory in practice. The authors suggest its use for fixing and determining oil content of feed water and boiler water samples as a referee method.

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Determination of Potash in Fertilizers or Base Goods

In the Absence of Ammonium Salts and Organic Matter

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THE proposed method for determining potash in fertilizers and base goods removes the calcium, iron, and aluminum as phosphates. after boiling in water, instead of removing the calcium as oxalate. Only one subsequent evaporation and no ignition are necessary; therefore there is no possible loss of K_2O by decrepitation or volatilization and the time and attention required in these operations are saved. In short, the determination is the same as in the case of potash salts, except that a little mono-, di-, or trisodium phosphate or phosphoric acid is added, followed by the addition of dilute sodium hydroxide until the solution is permanently alkaline to litmus.

A small amount of additional phosphate or phosphoric acid is needed to effect complete precipitation of the calcium, because calcium occurs in phosphate rock and superphosphate in excess of the P_2O_5 required to combine with it.

The method has been found repeatedly to yield very accurate and concordant results in the absence of ammonium salts and organic matter—e. g., in mixtures of superphosphate and potash salts. The test for the presence of ammonia may be made in an instant by simply adding sodium hydroxide to a hot aqueous solution of a portion of the sample and noting the odor. The appearance and odor of the sample itself will at once reveal the presence or absence of organic matter such as tankage, vegetable meal, etc.

The method is particularly useful because it is necessary for a manufacturer first to make a base when using considerable quantities of potassium sulfate, owing to the setting properties of the sulfate.

Five grams of the prepared sample are transferred to a 500-ml. flask, or 2.5 grams to a 250-ml. flask, and boiled for half an hour with approximately 350 or 200 ml., respectively, of distilled water; 30 ml. of a 2 per cent solution, or 6 ml. of a 10 per cent solution, of sodium phosphate are added, followed by dilute sodium hydroxide while the flask is whirled until the solution is permanently alkaline to litmus. If the solution again becomes acid on standing, more sodium hydroxide should be added, avoiding a large excess. The flask is then allowed to cool, the solution is made up to the mark, mixed, and filtered, and an aliquot of 25 ml., equivalent to 0.25 gram, is pipetted into a casserole or dish other than platinum; the platinic chloride and a few drops of hydrochloric acid are added, and the determination is completed according to the official method for K_2O in potash salts (*2*). Just before filtering, it is advisable to grind the precipitate lightly with a small pestle in order to remove excess platinum chloride and facilitate subsequent purification with ammonium chloride solution.

Table I reports determinations made to show the accuracy of the method.

Contamination amounting to 0.15 per cent due to dissolved silicates was found in the caustic soda in the case of base goods containing superphosphate made from Florida rock; therefore this amount was deducted. It is advisable to know the amount of dissolved glass in the sodium hydroxide used when not freshly made up and to make the necessary correction when the precipitate is not dissolved with hot water.

In analyzing the samples of base goods containing superphosphate made from Tennessee brown rock and triple superphosphate made from Idaho rock, fresh sodium hydroxide solution was made up from pure sticks; no silica whatever was found in the precipitates. All precipitates from these determinations were dissolved in hot water, and after drying, the Gooch crucibles were found to weigh the same as they did originally.

The strength of the alcohol used in all cases was 95 per cent. According to Scott (*2*) "too large a volume of alcohol should be avoided, as K_2PtCl_6 is slightly soluble in alcohol, especially that of 80 per cent. For this reason 95 per cent alcohol is preferable for the washing." Fresenius states that "potassium platinic chloride is not absolutely insoluble even in strong alcohol."

Since the Lindo-Gladding method of purifying the precipitate is effective, the presence of a small amount of lime does not interfere. For instance, 20.23 per cent K_2O was found in the sample of potassium sulfate base when all the calcium had not been precipitated, compared with 20.20 per cent when precipitation was complete.

When a large excess of platinum chloride is not added, some of the sodium will form sodium platinic chloride (Na_2PtCl_6) and some may remain as sodium chloride. In either case, the sodium is freed from the precipitate when the official method is followed. Equal weights of pure sodium chloride and pure potassium chloride were boiled in a flask with water, and slightly more platinum chloride was added to the aliquot than that required to combine with the potash; the result was 63.18 per cent K_2O as against 63.17 per cent theory. A large excess of platinic chloride is unnecessary. It is only necessary to have such excess as is readily shown by the color after evaporation, especially after the acidified alcohol has been added.

The dish or casserole should be removed from the steam bath soon after evaporation, in order to prevent the formation of insoluble compounds. The addition of a small amount of glycerol has been recommended, but the author has not investigated this point.

While the weighed portions of the samples were being boiled with water, they had a tendency to ball or lump, retarding or preventing complete extraction of the potash. In the case of the triple superphosphate base sample, lumping persisted throughout the entire half-hour boiling period. When the solution was made by adding alternately small portions of the weighed charge of the sample and water, and quickly giving the flask a whirl immediately after each addition of water, there was no caking during boiling. It appears that lumps of calcium sulfate, which is not

TABLE I. DETERMINATION OF POTASH

	K_2O Official %	K_2O Modified %
Base goods made in proportion of 1255 pounds of superphosphate and 745 pounds of C. P. K_2SO_4, having theoretical analysis of 20.13%. Superphosphate from Florida land pebble rock	20.02 20.05	20.20 20.21
Av.	20.035	20.205
Base goods made in proportion of 1255 pounds of superphosphate and 745 pounds of C. P. K_2SO_4, having theoretical analysis of 20.13%. Superphosphate from Tennessee brown rock	20.24 20.24	20.20 20.15
Av.	20.24	20.175
Base goods made in proportion of 1255 pounds of triple superphosphate and 745 pounds of C. P. K_2SO_4, having theoretical analysis of 20.13%. Triple superphosphate from Idaho rock	20.31 20.32	20.32 20.37
Av.	20.315	20.345
Potassium sulfate–magnesia base. Superphosphate from Florida land pebble rock	11.05 10.93	10.97 10.95
Av.	10.99	10.96

very soluble in either hot or cold water, are formed by the reaction between monocalcium phosphate and potassium sulfate. The fact that triple superphosphate is very high in monocalcium phosphate supports this view. The reaction may be expressed as follows:

$$Ca(H_2PO_4)_2 + K_2SO_4 = CaSO_4 + 2KH_2PO_4$$

A weighed portion is boiled with ammonium oxalate according to the official method to prevent loss by the action of the colloids, but in the case of high-analysis base goods, the potassium sulfate has the same effect, and complete recovery of the potash is obtained.

In making up the 20 per cent base mixtures, the same kind and amount of potassium sulfate were used in all cases. The slightly different results may largely be ascribed to differences in composition of the phosphate rock deposits. According to Jacob *et al.* (*1*), Florida land pebble phosphate contains 0.19 per cent K_2O (average of 11 analyses), Tennessee brown rock phosphate 0.435 per cent (average of 6 analyses), and Idaho phosphate 0.44 per cent (average of 3 analyses).

While the potash is originally present mainly as silicates, a small amount is probably decomposed by the action of fluorine in the mixing pan and during subsequent curing.

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Fluorometric Determination of Riboflavin in Pork Products

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A method developed for the fluorometric determination of riboflavin in pork and pork products has been applied successfully to ham, pork muscles, fat, liver, heart, lung, and spleen. It is in close agreement with other fluorometric procedures and the results do not differ greatly from values reported for the same tissues when analyzed by bioassay or microbiological procedures. The adsorption procedure of Conner and Straub and the permanganate oxidation step have been eliminated, thus shortening the time required for analysis.

Large differences were found in the riboflavin content of different muscles of the same pig. Appreciable amounts of riboflavin were found in various pork fats from which all lean had been removed.

THE most widely used procedures for the determination of riboflavin in meats and meat products have been the biological rat growth assay (*3, 7, 8, 10*) and the microbiological procedure of Snell and Strong (*15*). The latter method measures the influence of riboflavin on both the cell growth and the acid production of *Lactobacillus casei* ϵ grown on a synthetic medium free of riboflavin.

The fluorometric method, which measures the degree of fluorescence given by riboflavin in violet light, is being used with success in the analysis of many foods (*6, 9, 12, 16*), but its application to animal tissue has been only partially successful (*14, 18*). For many reasons, the application of fluorometric methods to all types of foods and biological materials seems desirable. All the proposed methods have certain limitations: The biological procedure is expensive and time-consuming and in some food materials there is the question of the availability of riboflavin (*11*). In the fluorometric method, excessive turbidity and the presence of extraneous fluorescent materials in solution are troublesome features. Extracts of certain biological material may exert either inhibitory or stimulating effects (*17*) in the microbiological procedure. Both the fluorometric and microbiological methods have in common the problem of bringing the vitamin into solution. As existing methods become refined, these and other difficulties will be overcome.

Mickelsen, Waisman, and Elvehjem (*13*), using the microbiological method, have made extensive investigations of the riboflavin content of meat and meat products. These workers point out that the values in the literature previous to 1934 on the distribution of riboflavin are not of a quantitative nature. Williams and the University of Texas group (*17*), using the same method, have made important contributions in studies of the vitamin content of tissues and foods. Of particular value to workers in this field is their demonstration of the variability in efficacy of extraction of the different vitamins depending on the nature of the food, the enzyme used, and the heat treatment involved.

Of the fluorometric procedures reported, that of Conner and Straub (*4*) appears to give reliable results with many plant products. This procedure was, therefore, taken as a starting point in the present studies, and the optimal conditions were determined for its application to pork products. The method which was finally devised differs from the Conner and Straub method in the following points: The adsorption procedure and permanganate oxidation steps have been omitted; the incubation period with clarase has been increased from two hours to 24 hours; and "blank" values are obtained by reduction of the riboflavin of the extracts with sodium hydrosulfite.

Description of Method

Because of the sensitivity of riboflavin to light, all manipulations are conducted in a semidarkened laboratory; and amber glassware is used throughout.

A 10- to 20-gram sample is dropped into 100 cc. of 0.04 *N* sulfuric acid in a Waring Blendor and macerated for 2 minutes at high speed. The creamy mixture is then transferred quantitatively to a 250- or 300-cc. Erlenmeyer flask, using a minimum amount of water from a wash bottle to effect the transfer. The flask is plugged with cotton and autoclaved 15 minutes at 6.8 kg. (15 pounds) pressure. As soon as the flask has cooled, 20 cc. of a 2.5 per cent solution of clarase (*4*) freshly prepared in a sodium acetate–acetic acid buffer are added. [The buffer solution (pH 4.5) is prepared by adding 54.4 cc. of glacial acetic acid to 66.9380 grams of anhydrous sodium acetate together with sufficient distilled water to obtain a solution of the reagents, and then transferred to a 1-liter volumetric flask and made up to volume with distilled water.] The contents of the flask are mixed thoroughly

and then incubated at a temperature of 45° C. for 24 hours. The flask is agitated two or three times during the incubation.

Following the incubation period, the extract is brought to a volume of 200 cc. At this point the contents of the flask should be thoroughly mixed, either by stoppering the flask and shaking thoroughly, or transferring the contents to a Waring Blendor and mixing for 30 seconds. The solution is then filtered and approximately 20 cc. of filtrate are collected. Clarification by centrifugation may be used instead of filtration, if preferred.

Into each of two test tubes, A and B, are pipetted 5 cc. of filtrate. Five cubic centimeters of water are added to tube A, and 5 cc. of an aqueous riboflavin solution containing 0.2 microgram per cc. to tube B. The solutions are mixed and fluorescent readings are made after adjusting the instrument. With the Coleman electronic photometer (Model 12), the instrument was adjusted each day with a sodium fluorescein solution of such strength that when its reading was 100, an aqueous solution containing 0.2 microgram of riboflavin read 70. This range was suitable for most pork extracts prepared in the above manner. Having obtained readings for A and B, 0.5 cc. of sodium hydrosulfite solution is added to each tube and readings are taken again. The average of these two readings constitute the reading of the sample blank, or C reading.

The hydrosulfite solution (*1*) was prepared by dissolving 5 grams of sodium hydrosulfite in 100 cc. of an ice-cold sodium bicarbonate solution (2 grams of sodium bicarbonate per 100 cc.).

To determine the concentration of riboflavin per cubic centimeter of extract, the following formula was used:

$$\frac{A - C}{B - A} \times 0.1 \text{ microgram per cc.} = \text{micrograms of riboflavin per cc.}$$

It is important that a "complete blank" containing all the reagents used in the method be run through the entire procedure, and the resulting concentration subtracted from the value of the sample extract. The high riboflavin content of clarase has not been sufficiently emphasized, nor has the fact that a considerable portion of the blank reading is due to the enzyme. Cheldelin, Eppright, Snell, and Guirard (*2*) have determined the riboflavin content of many of the enzymes. Clarase was not included in this group. In the proposed procedure, this enzyme contained 8.0 micrograms per gram. For pork muscle tissues its fluorescence reading represented 15 to 25 per cent of the total sample reading (A). Its blank reading (C), after reduction with hydrosulfite was large, representing 50 to 80 per cent of the values obtained for C readings of pork muscle extracts carried through the above procedure with 20-gram samples. In Table I are shown typical readings for pork muscle extracts.

Because of the remarkable clarity of the extracts, the time-consuming adsorption step of Conner and Straub was not used. Instead, aliquots of the extract were prepared by the procedure of Bailey and Thomas (*1*). The general practice of destroying colored impurities by oxidation with potassium permanganate was not resorted to, since independent experiments indicated that it was not permissible to assume that the blank value of any two permanganate-treated eluates would necessarily be identical, nor that they would necessarily be small and negligible. This is typical of many fluorometric procedures, as is also the assumption that the reading "increment" for a given amount of pure riboflavin will be the same for all permanganate-treated extracts of animal and plant tissues. The use of permanganate would also interfere with the sodium hydrosulfite reducing treatment subsequently used.

As emphasized by Bailey and Thomas (*1*), this method renders unnecessary the preparation of a standard curve of reference of photometer response unless one desires to know the extent to which the intensity of fluorescence of riboflavin is quenched by substances in the solution analyzed. The average quenching value of pork muscle extracts was 11.5 per cent, the low value of which shows the clarity of the extracts obtained by this method.

Table I. Typical Fluorophotometer Readings and Calculated Riboflavin Content of Pork Muscle Extracts

Sample	Fluorophotometer Readings[a] A	B	C	A-C	B-C	Calculated Riboflavin Content Extract	Muscle
						Microgram/cc.	*Microgram/gram*
1	58	91	12	46	33	0.139	2.58
2	53	86	10	43	33	0.130	2.40
3	55	87	11	44	32	0.138	2.56
4	67	97	11	56	30	0.186	3.52
5	40	72	10	30	32	0.094	1.68
Enzyme blank	10	40	7	3	30	0.010	....

[a] A, reading of extract.
B, reading of extract containing 1 microgram per cc. of added riboflavin.
C, reading of extract after reduction with sodium hydrosulfite.

Table II. Effect of Time of Incubation with Clarase on Yield of Riboflavin from Pork Muscle

Sample	2 Hours	24 Hours
	(*Micrograms/gram of fresh pork tissue*)	
A	1.67	2.26
	1.98	2.28
B	1.57	2.12
	1.70	2.34
C	1.58	2.24
	1.74	2.34
Average of 12 loin samples	1.32	1.78

Critical Study of Steps in Procedure

Extraction Method and Enzymatic Hydrolysis. In preliminary determinations, the method of extraction by Conner and Straub (*4*) was used with modifications. In all direct comparisons adjacent slices of a single, separated muscle were used for samples. Samples of pork muscle which had been mixed in 0.04 *N* sulfuric acid with a Waring Blendor were digested for varying lengths of time on a boiling water bath previous to incubation with clarase. These results were compared with analyses of the same samples which had been autoclaved for 15 minutes at 15 pounds' pressure. The results of these experiments indicated that the length of heating was not so critical a factor as the length of time of incubation with enzyme subsequent to heating. Thus, 12 duplicate samples of loin gave an average value of 1.75 micrograms of riboflavin per gram when heated for 1 hour on a boiling water bath, as compared to an average value of 1.78 micrograms per gram when the samples were autoclaved. (These samples were subsequently incubated 24 hours with clarase.) In the routine determination of a large number of samples it proved to be more convenient to use the autoclave throughout, with results from check samples agreeing more consistently.

Incubation of samples with clarase for 2 hours as in the method of Conner and Straub gave erratic results and the values were frequently considerably lower than those which had been reported in the literature (*13*) for similar products analyzed by the microbiological method. Also, the exhaustive investigations on the enzymatic liberation of B vitamins from plant and animal tissues by Cheldelin *et al.* (*2*) suggested that a 2-hour incubation might not be sufficient. This proved to be the case. In Table II are shown the values for a 2-hour and a 24-hour incubation with 2.5 per cent clarase (incubation for more than 24 hours did not improve the yield). Values range from 15 to 35 per cent greater for the 24-hour incubation. These figures also show the duplicability of results by this method for pork products.

Since Cheldelin *et al.* (*2*) had demonstrated that enzymes may differ widely in the efficiency with which they will liberate riboflavin from foods, it seemed desirable to compare the effectiveness of other enzymes with that of clarase. Solutions (2.5 per cent) of clarase, papain, takadiastase, and mixtures of the latter two were used on aliquot samples of pork muscle exactly as described for clarase in the original method. Relative yields for these enzymes were: clarase 100 per cent, papain 49 per cent, takadiastase 81 per cent, and papain and takadiastase 54 per cent. It is not intended to imply that these are the maximum yields obtainable with the enzymes other than clarase. A variation in conditions might easily change the order of efficacy. These results do, how-

Table III. Comparative Results Obtained by Proposed and Conner and Straub Adsorption Procedures

Sample No.	Proposed Method *Micrograms/g.*	Adsorption Method *Micrograms/g.*
12	2.54	2.50
13	1.82	1.96
16	1.74	2.08
20	1.82	1.90
28	1.92	2.00

ever, represent the values obtained under the conditions of the proposed method. The Texas group (*17*) usually obtained maximum yields of riboflavin from tissues with papain or combinations of papain and takadiastase.

Comparison of Results by Different Fluorometric Procedures. In order to test the method it seemed desirable to compare the results obtained with those of other methods which had been suggested. Good agreement was found with the Van Duyne (*18*) procedure in the case of pork heart or liver, but poor checks were obtained with muscle tissues. In the Van Duyne method the sample is digested for 24 hours with 1 per cent pepsin in hydrochloric acid at 37° C. The lack of provision for determining the size of the increment due to pure riboflavin in the Van Duyne procedure and the relatively low concentration of riboflavin in muscle tissue with its concomitant high blank value probably account for the lack of agreement.

A comparison of the proposed method with the results obtained by the adsorption procedure of Conner and Straub is shown in Table III. These values are averages of duplicate determinations of pork tenderloin muscles. The extracts adsorbed on Supersorb were those obtained by the extraction procedure described.

Ordinarily the adsorption method yielded slightly higher results. It is possible that this can be accounted for by the assumption which is implied in the Conner and Straub procedure that the blank value of the test material extract after adsorption and permanganate treatment is negligible.

Good agreement was also obtained on pork extracts with the Najjar (*14*) method proposed for the fluorometric determination of riboflavin in urine and other biological fluids. The Najjar method involves treatment of the extract with permanganate, followed by peroxide and subsequent extraction of the riboflavin by use of butyl alcohol. Pyridine and sodium sulfate are added before extraction with the alcohol. The blank value is obtained by exposing the sample to a mercury vapor lamp (or direct sunlight) for an hour or two. Six cured ham samples gave an average value of 2.08 micrograms per gram by the proposed method and 1.85 micrograms per gram by the Najjar method.

Table IV. Riboflavin Content of Pork Muscles from the Same Pigs

(Micrograms per gram of fresh tissue)

Muscle	Riboflavin Content
Shoulder (triceps-Brachii)	3.0
Tenderloin (Psoas Major)	2.4
Loin (anterior end; Longissimus Dorsii)	1.3
Loin (posterior end; Longissimus Dorsii)	1.3
Ham (inside; adductor)	1.3
Ham (eye; Semitendinosus)	1.8

Riboflavin Content of Pork and Pork Products. The method has been applied to various pork muscles, fat, organs, and hams, both dry-cured and brine-cured.

In Table IV are shown riboflavin values for various fresh pork muscles. The muscles were obtained from two pigs which had been fed a standard fattening ration, and samples from the right and left sides of the carcass were taken as duplicates. Each value represents the average of two duplicate determinations (one duplicate for each pig). The high values for shoulder and tenderloin muscles, and the large differences between different muscles were unexpected. Such differences in the muscles themselves may be significant in evaluating published results.

Table V gives the values obtained from various pork organs compared with data available on similar organs from the work of Mickelsen *et al.* (*13*). Cheldelin (*2*) reports a somewhat lower value, 8.4 micrograms per gram for pork heart. Darby and Day (*5*), using the rat bioassay method, give values of 3.0 micrograms per gram for fresh ham and 23.0 micrograms per gram for pork liver. The high value of 0.9 microgram per gram reported by the latter workers for bacon suggested that either the bacon studied by them was unusually high in its content of lean, or pure pork fat itself contained significant amounts of riboflavin. This prompted a study of the various types of pork fat, the results of which are presented in Table VI. The relatively high values obtained are important in view of orthodox concepts regarding the solubility characteristics of riboflavin. These findings suggest a reconsideration of animal studies concerned with the "sparing" action of fats on the B vitamins.

Table V. Riboflavin Content of Various Pork Organs

(Micrograms per gram of fresh tissue)

Organ	Values by Proposed Method	Values by Mickelsen *et al.*
Liver	22.0	25.4
Liver	29.4	26.9
Liver	....	29.0
Heart	10.1	11.2
Heart	9.1	....
Lung	3.4	....
Lung	2.7	....
Spleen	3.6	....
Spleen	4.3	....

Table VI. Riboflavin Content of Pork Fats

Type of Fat	Riboflavin Content *Microgram/g.*
Lard	0.09
Ham facing	0.63
Back fat	0.59
Leaf fat	0.57

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An Improved Pressure-Regulating Device

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A manostat capable of controlling a large range of pressures over a large range of flows is described. The method of operation is given and the limiting conditions for its use are stated in terms of mathematical equations and graphs.

EXPERIMENTS in progress necessitated the use of a manostat capable of controlling a large range of pressures over a large range of flows. The problem was to pass a gas through a reaction chamber at definite pressures and at varying rates of gas flow. As the gaseous products must be undiluted with other gases when collected on the low-pressure side, a foreign gas or air-leak device could not be used. A simple and accurate device for such a purpose has not been described in the literature. Caldwell and Barham (*1*) describe a float-valve manostat for high pressures, while Lewis (*2*) depicts an accurate device for low-pressure control based upon the throttling action of a sintered-glass disk in contact with mercury. Both these systems are self-contained but have their limitations in pressure and flow ranges.

In the present method, a sintered-glass disk, a by-pass controllable needle valve, and a mercury column which also serves as a manometer are used on the high-pressure side, and a throttling needle valve on the low-pressure side. In Figure 1 the system is schematically shown.

m is a mercury column connected by three-way stopcock *f* either to the mercury reservoir *i*, or to the system whose pressure is to be controlled. *d* is a sintered-glass disk that allows gas, but not mercury, to pass. This disk is slightly inclined to the mercury column in contact with it, so that it may be only partly covered. *c* is a check valve to prevent mercury from passing into the system in case of a sudden decrease in the pressure in the system. *a* and *b* are stainless-steel needle valves wherein the movement of the needle is effected by a bellows. *e* is a trap to collect mercury which may distill through the disk.

In operation the desired pressure for the system is set by connecting mercury reservoir *i* and manometer *m* by means of three-way stopcock *f*; mercury is added to or withdrawn from *m* through the use of stopcock *g*. Stopcock *f* is then turned to connect *m* with the system, and valves *a* and *b* are adjusted until the sintered-glass disk *d*, is partly covered with mercury. The pressure in the system is then measured from *h*, which is the difference in the levels of the mercury in *m* and at *d*. It is necessary to throttle valve *a* only at very low flows through the system. Valve *b* is used at large flows, since the gas will flow through *d* at small rates only because of its high resistance to gas flow. When the pressure in the system is higher than the desired value, the sintered-glass disk is uncovered and gas flows through the disk to the vacuum side, whereas, when the pressure is low, the disk becomes covered with mercury and gas does not pass through, thus allowing the pressure to build up.

Figure 2 shows the maximum rates of flow of air through *d*—that is, with *b* closed. These values depend to a certain extent on the capacity of the pump in the vacuum system. Although the medium-grade filter has the advantage of low flow resistance, its use is limited to pressure differentials up to 1.5 atmospheres, at which point mercury passes through the disk. The fine-grade disk was tested up to 2 atmospheres' pressure differential, and no passage of mercury through it was observed.

The principal advantage of this type of control over the float method is that the problem of the relationship between the weight of the float and the pressure differential is eliminated. One sintered-glass disk functions at all pressures until the pressure differential is reached where mercury passes through the disk.

Certain limitations in the use of a sintered-glass disk in a manostat are imposed by passage of mercury through the disk and restriction of gas flow.

The relation governing the passage of mercury can be shown to be

$$2\,\gamma\cos\theta = -(p_1 - p_2)r \qquad (1)$$

where γ = surface tension of the mercury
$p_1 - p_2$ = pressure differential across the disk
r = radius of pores (average)
θ = contact angle

That for flow resistance can be closely approximated by either the Poiseuille equation

$$G = \frac{\pi(p_1 - p_2)r^4}{8\eta d}\,\frac{M}{RT}\,p \qquad (2)$$

or the Knudsen equation when the mean free path is comparable with *r*:

$$G = \frac{4}{3}\sqrt{2\pi}\,\sqrt{\frac{M}{RT}}\,\frac{r^3}{d}\,(p_1 - p_2) \qquad (3)$$

where G = mass of gas flowing through per second
η = viscosity of gas
p = average pressure in the disk
M = molecular weight of gas
R = gas constant
T = absolute temperature
d = thickness of disk

and the other terms are as defined for Equation 1.

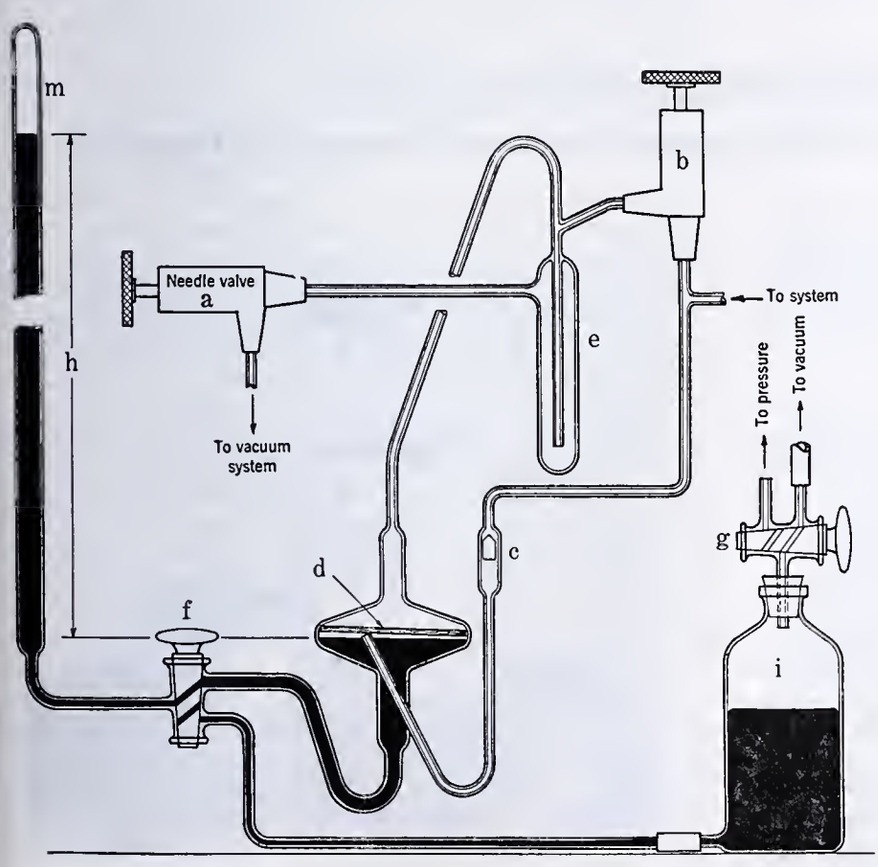

FIGURE 1. APPARATUS FOR CONTROL OF PRESSURE AND FLOW OF GASES

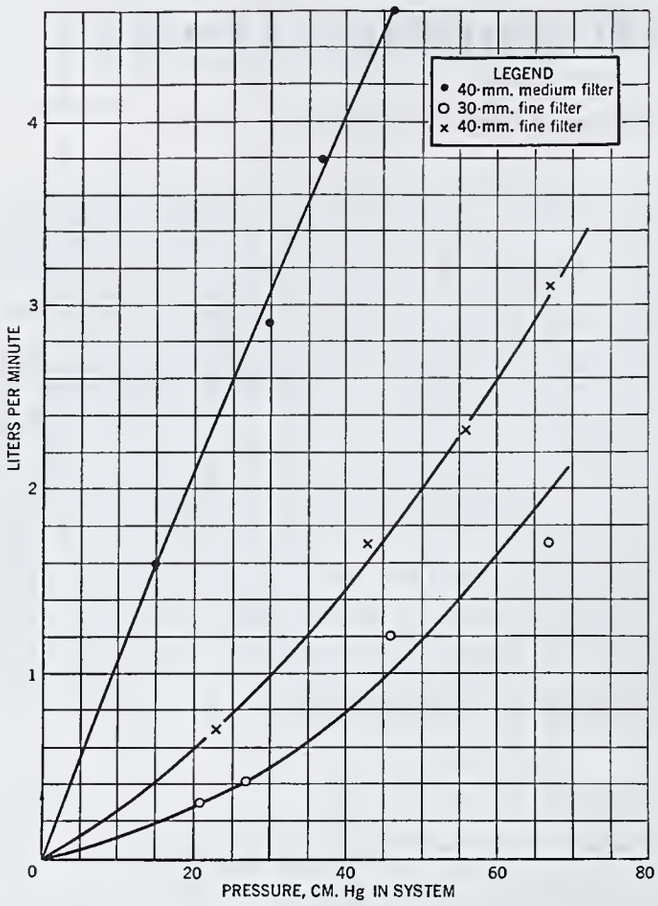

FIGURE 2. MAXIMUM RATES OF GAS FLOW THROUGH SINTERED-GLASS DISKS

As can be seen from these relations, the limiting pressure differential for nonpassage of mercury varies inversely as r, while the flow of gas varies as the cube or 4th power of r. It follows, therefore, that a disk with low resistance to flow will also have a low resistance to passage of mercury, which destroys the regulating function of the disk.

For example, the medium disk passed mercury at about 1.5 atmospheres' pressure differential. Using the following values in Equation 1

$$\gamma = 485 \text{ dynes per cm.}$$
$$p_1 - p_2 = 1.5 \times 10^6 \text{ dynes per sq. cm.}$$
$$\cos\theta = -1$$

r is calculated to be about 6.6 microns. The fine disk was tested up to 2 atmospheres without passing mercury. From this one would estimate r to be of the order of 3 microns.

While this disk can be used at greater pressure differentials, the gas flow, as shown by Equations 2 and 3 and by Figure 2, is considerably reduced. To obviate this difficulty valve b was inserted in parallel with the disk. This valve can be adjusted to the point where the flow of gas to the disk is of a magnitude capable of being handled by the disk and the regulating property of the disk functions.

Because of its ready availability a fritted-glass disk was used. A much better scheme would involve the use of a long, cylindrical cup of fritted glass, whereby the mercury would find its level by covering or uncovering the cylinder for passage of gas. This was done by Wansbrough-Jones (*3*), who used a clay tube closed at one end for regulating flow of gases at very low pressure differences. In this case, however, the presetting of a desired pressure would be more difficult since d, Figure 1, would no longer be a fixed point and height h would be a variable. Pyrex brand sintered-glass disks manufactured by the Corning Glass Co. were used.

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Modified Hershberg Melting-Point Apparatus

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ACCURATELY controlled temperature and heating of melting-point baths have long been a problem in the determination of melting points up to about 150° C. Where the temperature is controlled by heating resistance units, one is apt to encounter a lag or overshoot in the temperature when contact is made or broken.

The Hershberg precision melting-point apparatus[1] has been modified, so that the melting-point temperatures of low-melting solids may be maintained to within 0.1° C. over a longer period of time (Figure 1). Heat is applied through radiation from an infrared drying bulb of 105 to 120 volts and 250 watts. When the lamp is turned on or off, heat is applied or discontinued instantaneously, thus avoiding any heat lag or overshoot. The bulb is placed within a metal container sufficiently ventilated to allow for heat dissipation and is focused on the heating chamber of the melting-point bath. The chamber consists of a flattened portion of the Thiele tube made by sealing two 6-cm. Pyrex petri dishes into the tube. By using the infrared bulb specified the limiting temperature is about 150° C. A higher temperature could be attained by using two heating units, one on either side of the heating chamber, or by using lamps of higher wattage. The heating is controlled by means of a variable-voltage transformer.

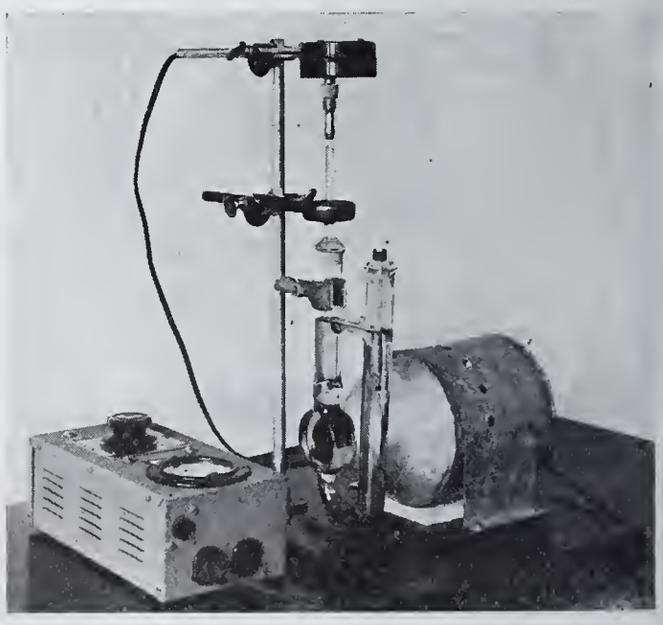

FIGURE 1

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Hydrogen Electrode Half-Cell in Polarography

J. PERCY BAUMBERGER AND KATHLEEN BARDWELL

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The hydrogen electrode is shown to serve as an adequate and convenient anode in polarographic work. The fact that the potential of the anode may be changed at will to potentials between *Eh* 0 and −0.8 by simply adjusting the pH of the hydrogen electrode solution makes it possible to change the applied voltage to values that will give the optimum definition and separation of polarographic waves. Some improvements of the Sargent type of polarograph are suggested.

IN POLAROGRAPHIC work the position and height of each wave on the current-voltage curve are usually determined (*9*). The maximum clarity of these waves can be obtained by adjusting the voltage to make the relation of the scale of abscissas (voltages) to ordinates (amperes) such that the waves are properly accentuated in form and location. In this paper it is shown that the attainment of this objective is facilitated by the use of the hydrogen electrode as an adjustable anode or as a reference half-cell. The use of the normal hydrogen electrode as final reference is, of course, implied in polarographic as well as in all electrochemical work, but the author has not found a published statement of its actual use as a half-cell with the dropping mercury electrode. Müller (*8*) states "The hydrogen electrode has been used in Prague by myself and others. The ease by which its potential can be altered up and down the scale has never been pointed out."

It is the usual procedure (*4, 5, 7*) to leave a large pool of mercury on the wide bottom of the conical electrode vessel of the polarograph to serve as the quiet, difficultly polarizable half of the cell, while the dropping mercury electrode serves as the easily polarizable electrode. The actual potential of the quiet electrode may be determined by connecting it to a reference half-cell—e. g., a calomel half-cell. It is essential that the value be thus checked because the potential of the quiet electrode will vary with the electrolyte in the electrode vessel.

Müller and Baumberger (*9*) used the saturated calomel half-cell directly and avoided the use of the large quiet mercury reference. Thus they had a convenient constant reference point for the calculation of potentials and were saved the inconvenience of using relatively large quantities of mercury, as well as the trouble of filling side arms with mercury, etc.

However, the saturated calomel half-cell has a rather high value, $Eh = 0.246$ at 25°. Since many of the determinations made with the polarograph are carried out at relatively low *Eh*—e. g., protein waves at $Eh = -1.2$ volts—the potential difference between a calomel half-cell and the dropping mercury electrode may be of the order of 1.5 volts. The applied voltage on the dropping mercury electrode is calculated from the position at which the bridge is tapped, the bridge voltage, and the reference half-cell value. It is obvious that if the *Eh* of the reference half-cell approaches the *Eh* of the dropping mercury electrode, the bridge voltage can be diminished and a given change in the position of the sliding contact of the bridge resistance will correspond to a much smaller *Eh* change on the dropping mercury electrode.

In the polarographic apparatus, as usually available, the bridge span corresponds to an exposure of the full length of the photographic recording surface, usually with abscissas flashed automatically at ten or twenty intervals. By varying the voltage across the bridge it is possible to make these abscissas correspond to suitable *Eh*. When the bridge wire is short, using a small bridge voltage gives increased accuracy.

In the Nejedly polarograph resistances are so arranged that they may be added before, after, or at both ends of the slide wire, thus placing the slide wire in a position in the bridge in which it may be most effectively used to adjust the applied voltage.

The voltage span of interest in the polarograph of a particular compound might be −1.0 to −1.3. In such a case, if a reference half-cell of $Eh = -0.9$ were used, the bridge voltage could be made 0.5 volt and the polarographic waves would be stretched out on the horizontal axis of the graph in such a way as to give suitable detail. Clark (*3*) and Kolthoff and Lingane (*5*) list many reference half-cells of various high and low potentials and one of these could be chosen—e. g., Hg/HgS, −0.77. Instead of using a number of different types of anodes, the advantages described above can be obtained by using a hydrogen electrode as anode and adjusting the pH of its solution to such a value that the desired *Eh* will be obtained. At 25° C., $Eh = -0.059$ pH. In practice a very simple hydrogen electrode half-cell serves the purpose adequately.

A 2-inch piece of No. 30 platinum wire is sealed into soft glass, so as to permit a mercury contact to the copper leads, and is then platinized. A four-hole stopper fitted to a shell vial carries a fine inlet bubbler for hydrogen, an outlet through a water valve, a saturated potassium chloride agar-agar salt bridge, and the platinized platinum electrode. The vessel is half filled with a buffer of appropriate pH and hydrogen gas is bubbled vigorously and continuously through the solution. This hydrogen electrode half-cell is connected by the salt bridge to a saturated potassium chloride connecting vessel, which in turn is connected to the dropping mercury electrode half-cell by a second salt bridge, completing the chain. Hydrogen gas

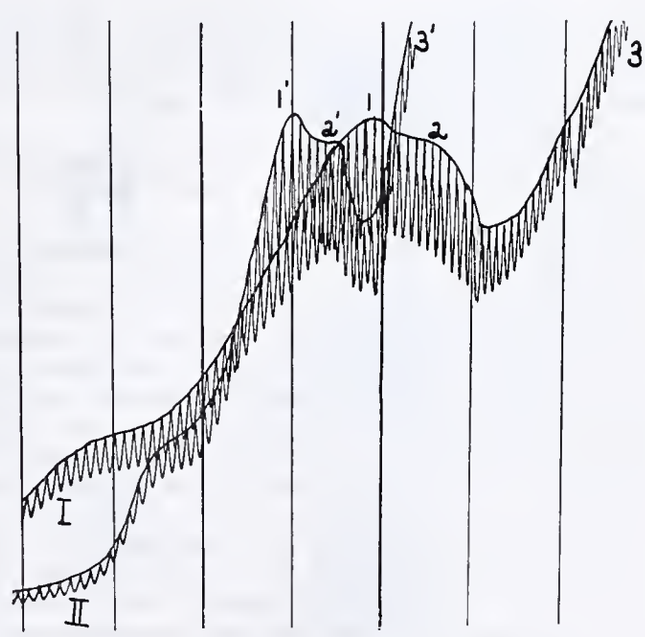

FIGURE 1. SEPARATION OF POLAROGRAPHIC WAVES BY CHANGING APPLIED VOLTAGE

A line has been drawn along crests of oscillations.
Material. Pemphigus bulbous fluid in $CoCl_2$, NH_4OH, NH_4Cl.
Conditions. Anode, curve I, hydrogen electrode at pH 6.8, $Eh = -0.408$. Curve II, quinhydrone electrode at pH 6.8, $Eh = 0.287$.
Volts/abscissa. I = −0.15, II = −0.30.
Initial applied voltages. I = −0.45, II = −0.90.
Temperature, 30° C. Sensitivity, 1/50.

[1] Present address.

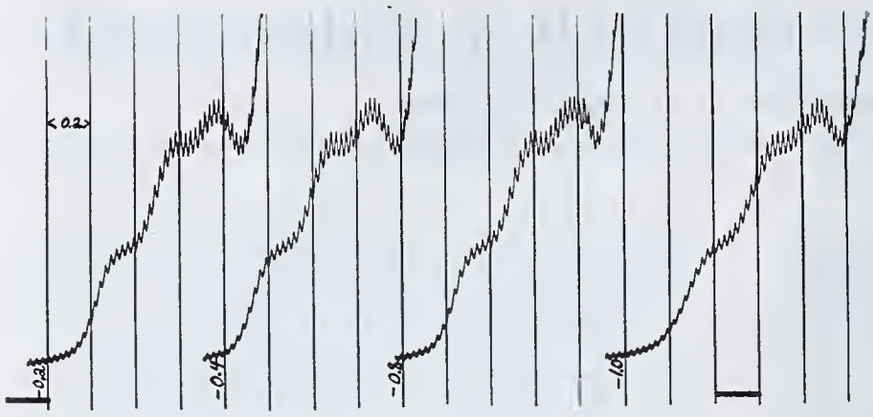

FIGURE 2. EFFECT OF NATURE OF ANODE ON POLAROGRAM

Material. Human blood serum.
Anodic half-cells. Curve 1, hydrogen electrode at pH 6.8. Curve 2, hydrogen electrode at pH 4.71. Curve 3, saturated calomel half-cell. Curve 4, quinhydrone at pH 6.8.
Temperature, 22° C. Sensitivity, 2/300. Applied voltage, 3.8.
Initial applied voltage is indicated on each curve and abscissas indicate increasingly negative voltages for each curve at 0.2 volt per abscissa.

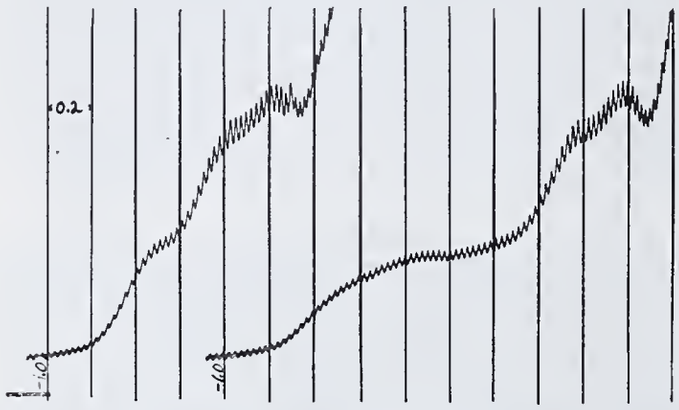

FIGURE 3. EFFECT OF STIRRING SOLUTION IN QUINHYDRONE ANODE

1, stirred. 2, unstirred.
See caption of Figure 2 for other details.

from a tank of the commercial product, passed through a wash bottle, gives a potential in the neighborhood of the expected potential calculated from the pH of the buffer. This potential remains very constant and may be readily determined potentiometrically, using the calomel half-cell for reference.

If suitable precautions (*3*) are taken, the hydrogen electrode potential may be calculated directly from pH and serve as an absolute reference point.

The extension of polarographic waves on the horizontal axis by changing the applied voltage is illustrated in Figure 1, where the bridge voltage is twice as great in II as in I. The material used to illustrate these points is in Figure 1 a blister fluid, pemphigus bulbous fluid (*6*), and in the other figures human blood serum. In each case the protein-containing fluid is added to a cobalt chloride–ammonium hydroxide–ammonium chloride mixture which forms a complex of Co^{++} and the cysteine or cystine of the protein (*2*). The resultant solution gives a polarogram having three curves: first the reduction of Co^{++}, and then the catalytic reduction of the hydrogen of the metalloprotein complex, giving a pair of waves labeled 1, 2 and 1′, 2′, and finally the reduction of Na^{+} labeled 3, 3′ in Figure 1. In the subsequent figures the waves are not labeled but are in the same sequence.

Figure 1 shows that waves 1, 2, and 3 are separated twice as far from each other as are 1′, 2′, and 3′, and the *Eh* at which each occurs can therefore be more accurately determined. This separation of the wave would not be possible if an anode of high potential, such as the saturated calomel half-cell, were used because the voltage necessary would be about 2.2, whereas with a hydrogen electrode as anode the voltage could be halved.

The apparatus used to obtain Figure 1 was changed to fulfill the requirements of this type of investigation.

The Heyrovský Polarograph, Model XI, manufactured by E. H. Sargent & Company, Chicago, was used. It was found helpful to modify this instrument in four ways: (1) Ten contacts are placed on the single-revolution slide wire, so that the electric circuit of a lamp is completed ten times in the span of the bridge voltage. This light illuminates the entire slit of the photographic drum and records abscissas, as shown in Figure 1. Before this modification the applied potential could be indicated only by calculation from one end of the photographic record.

(2) The photographic drum of this make of polarograph has its entire circumference calibrated into ten equal sectors. These, however, do not correspond to tenths of the applied voltage, since the slide wire of the bridge makes only a partial revolution and is geared to the photographic drum directly. This was remedied by recalibration, so that definite fractions of the drum's revolution corresponded to tenths of the bridge. Thus the correspondence of slide wire contact position and recording cylinder reading, so conveniently available in the Nejedly polarograph, was obtained.

(3) No provision is made in this make of apparatus for working with fixed applied voltage to measure the change in concentration with time of some electrolyzable substance as, for example, the utilization of oxygen by yeast cells (*1*). A switch is arranged, so that the moving contact of the slide wire is out of the circuit and the applied voltage is read directly from the voltmeter. The revolving drum then serves to record the changes in diffusion current occurring with time.

(4) Although the galvanometer of this make of apparatus is very stable, the vibrations of the street caused considerable vibration, as did also the process of loading and unloading the photographic drum. This was overcome by supporting the galvanometer on a 0.25-inch metal bracket entering the case of the apparatus through a slit and fastened to the outer brick wall of the building. The case of the apparatus was then fastened to a fixed table, so that the relation of galvanometer to optical system would remain constant. (All the above changes were made by Harry Fulbright, Physics Department, Washington University, and the author wishes to express appreciation of his good workmanship and helpful advice.)

The adequacy of the hydrogen electrode as anode is shown in Figure 2. Here polarographic curve 3 with a calomel half-cell as anode agrees perfectly with curves 1 and 2 in which hydrogen electrodes of different pH are employed as anodes.

The quinhydrone electrode does not serve as a perfectly satisfactory anode, apparently becoming somewhat polarized, as indicated by the fact that the polarogram is stretched out over a wider voltage range than is the case when the hydrogen electrode

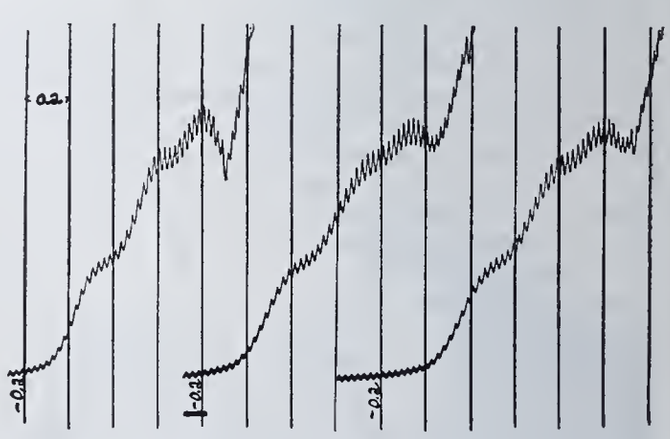

FIGURE 4. EFFECT OF CONCENTRATION OF BUFFER ON HYDROGEN ELECTRODE AS ANODE

Phosphate buffer of pH 6.8 used in all cases. Logs of molar concentrations of buffer; curve 1, −1; curve 2, −3; curve 3, −5.
See caption of Figure 2 for other details.

or calomel electrode is used as anode. This is shown in Figure 2 by comparison of curve 4 with curves 1, 2, and 3. Stirring greatly improves the performance of the quinhydrone electrode as anode, as is shown by Figure 3, where the unstirred electrode as anode gives a polarogram more drawn out than the stirred.

The concentration of buffer necessary to make a hydrogen electrode a sufficiently stable anode is suggested by the results of dilution of buffer shown in Figure 4. Here phosphate buffer of pH 6.8 is satisfactory when log $M = -1$ but allows the polarogram to be distorted where log $M = -3$ and even more so when log $M = -5$.

Since the hydrogen electrode is perfectly reversible, its merits as an anode also apply to its use as a cathode when the dropping mercury electrode is anodic.

Summary

The hydrogen electrode may be used as anode (or cathode) in polarographic work, if it is sufficiently well buffered to remain unpolarized. The quinhydrone electrode, even when rapidly stirred, is less satisfactory for this purpose.

The relatively low potentials obtainable with the hydrogen electrode permit the use of low bridge voltages and consequently the spreading of polarographic waves.

The hydrogen electrode potential can be readily adjusted by changing the pH of the solution in which it is immersed; this makes it a convenient half-cell for polarography.

Several changes have been made which add to the usefulness of the Sargent type of Heyrovský polarograph.

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Automatic Pressure-Regulating Manometer

ROGER GILMONT AND DONALD F. OTHMER, Polytechnic Institute of Brooklyn, Brooklyn, N. Y.

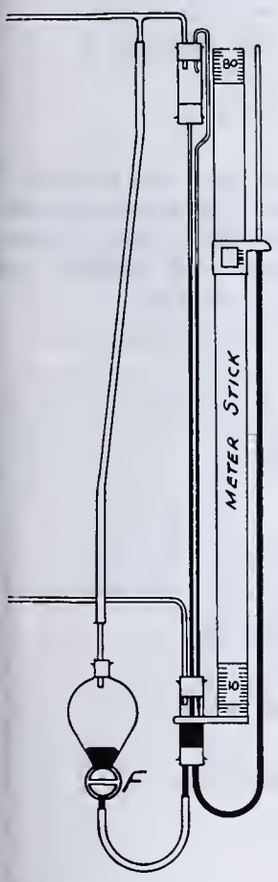

FIGURE 1. GENERAL ARRANGEMENT OF MANOMETER AND CONSTANT PRESSURE DEVICE

Upper open line goes to source of vacuum, lower open line goes to system, both preferably by way of traps to catch any mercury pulled through lines.

THE device shown in Figures 1 and 2 was developed in the experimental study of the vapor-liquid equilibria of the binary system, water–acetic acid, at subatmospheric pressures (*1*), using the equilibrium still previously described (*2*). It has also proved of use in controlling pressures in other operations, such as vacuum distillations. This pressure regulator contains no moving parts. It can be constructed without glass blowing and includes a direct-reading manometer.

A meter stick measures the height of the mercury column in the closed-tube manometer above the mercury level in the trap. A movable indicator with a vernier reads the mercury level in the manometer tube when the meter stick is adjusted so that a fixed indicator reads the level in the lower trap. Two slots in the meter stick fitting over screws with thumb nuts enable vertical adjustment. With the manometer tube filled with mercury and the level of mercury in the lower trap below the opening of the tapered tip of the right-hand tube, suction is applied. The level in the lower trap is adjusted by means of the mercury reservoir—a standard separatory funnel. When the level of the mercury in the manometer falls to the desired point, the level of the mercury in the lower trap is adjusted so that a seal is just made with the

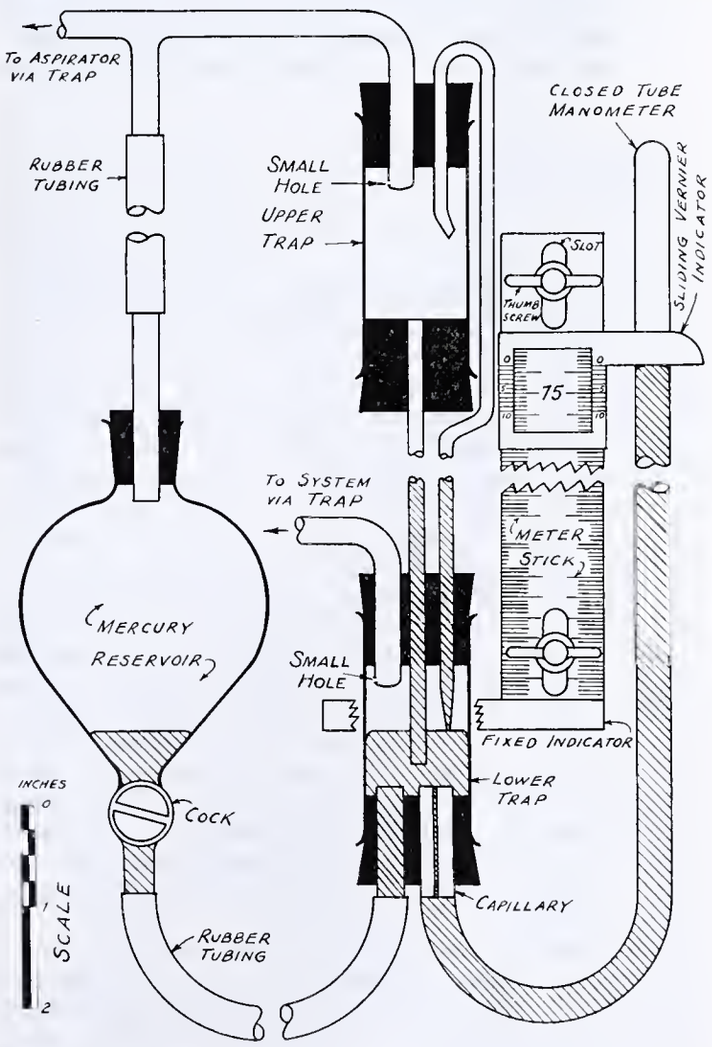

FIGURE 2. DETAILS OF MANOMETER AND CONSTANT-PRESSURE DEVICE

opening of the tapered tip, thereupon the stopcock of the reservoir is closed. The lower trap then cuts off suction of gas through the right-hand tube. If the system were perfectly gas-tight at constant temperature, the pressure would remain constant and the pressure regulator would be unaffected. If air does leak in, mercury rises in the manometer and the two other tubes to depress the level in the lower trap, so that the higher pressure of the system causes gas to rise through the right-hand tube, carrying the mercury with it. The mercury returns to the lower trap by way of the left-hand tube. Gas is withdrawn by the evacuator until the pressure and mercury drop and the level is restored in the lower trap to its original position, at which further evacuation is prevented by the seal.

The regulator maintains constant pressure within about 2 mm. at lower pressures and within about 5 mm. at pressures approaching atmospheric. By using vertical tubes of smaller bore, the regulator can be made more sensitive. The manometer can be read to approximately 0.1 mm., so that in distillations the corresponding temperature should be read simultaneously with the pressure, when the pressure drifts to the desired value. Since vapor-liquid equilibria are insensitive to changes in pressure as large as 10 mm., the control of the regulator is sufficiently precise for this work as well as for most vacuum distillations.

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Apparatus for Photoelectric Titrations

Application to Dark-Colored Resins

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A photoelectric instrument, generally applicable to acid-base and oxidation-reduction titrations employing colored or fluorescent indicators, is described. It is especially useful for titrations which cannot be carried out satisfactorily by the usual visual or electrometric means. The application of this instrument to the determination of acid and saponification numbers of dark-colored resins is discussed in detail. A precision of ±1 per cent is easily obtained.

Preliminary work has shown that precipitation reactions and reaction rates can be studied, and, in addition, the apparatus may be used as a chemical colorimeter.

THE first objective measurement of the color change of an indicator as a means of determining the end point of a titration was made in 1926 by Field and Baas-Becking (*7*), who applied it to the starch-iodine reaction. These workers used a radiomicrometer with a receiver consisting of a silver-bismuth thermocouple. In 1928, Müller and Partridge (*33*) described a photoelectric apparatus for automatic titrations employing a single vacuum phototube, an amplifier, a relay, and an electromagnetic buret release. This equipment was used for acidimetry and alkalimetry, permanganate, dichromate, and iodometric titrations. Furthermore, it was found that certain precipitation reactions, such as the determination of chloride ion by silver ion in the presence of chromate ion, could be followed. The precision in every case was greater than that of visual estimation.

During the past decade, several papers have been published by Hirano (*13–24*), Somiya (*44–47*), Kasai (*26*), and their co-workers in Japan describing the application of photoelectric titration to various quantitative procedures. During the same period, Ringbom and Sundman (*40*) and Müller (*29*) in Germany, del Campo and co-workers (*3*, *4*) and Gonzales (*9*) in Spain, and Lur'e and Tal (*28*) in Russia have made contributions to the subject. In this country, Alyea (*1*), Boyer (*2*), Goodhue (*10*), Hickman and Sanford (*12*), Müller and his co-workers (*30–34*, *37–39*), Rowland (*41*), Russell and Latham (*42*), and Styer (*48*) have described improvements in photoelectric titration technique.

In the authors' laboratories electrometric titrations have been used in the past where visual end points with colored indicators are difficult or impossible to obtain. However, in certain cases, electrode equilibrium is slow and titrations are time-consuming. For example, in applications involving two-phase systems, or in those carried out in certain organic solvent media, it is sometimes necessary to wait for 10 to 20 minutes after each addition of titrant before equilibrium is established. Furthermore, in these cases, the electrometric end point is often not very sharp, whereas indicator color changes sometimes remain sharp, although they are often obscured by background color or turbidity.

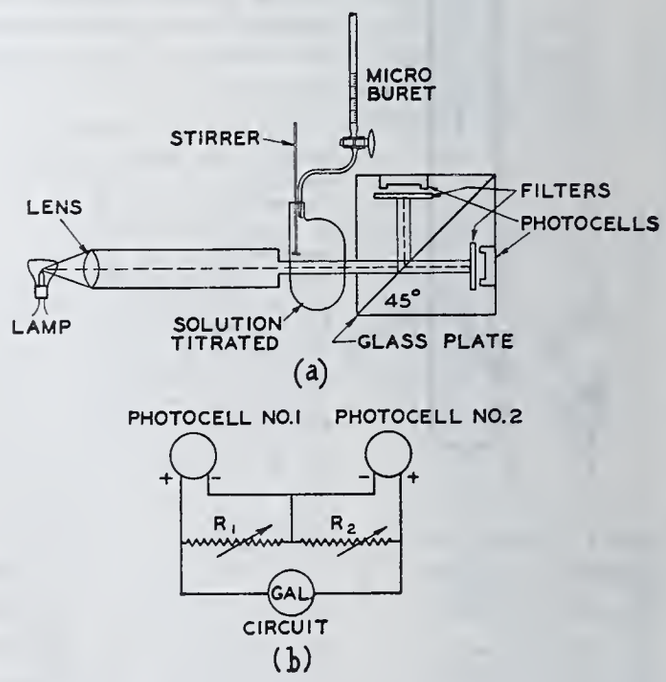

Figure 1. Schematic Diagram of Photoelectric Titration Apparatus

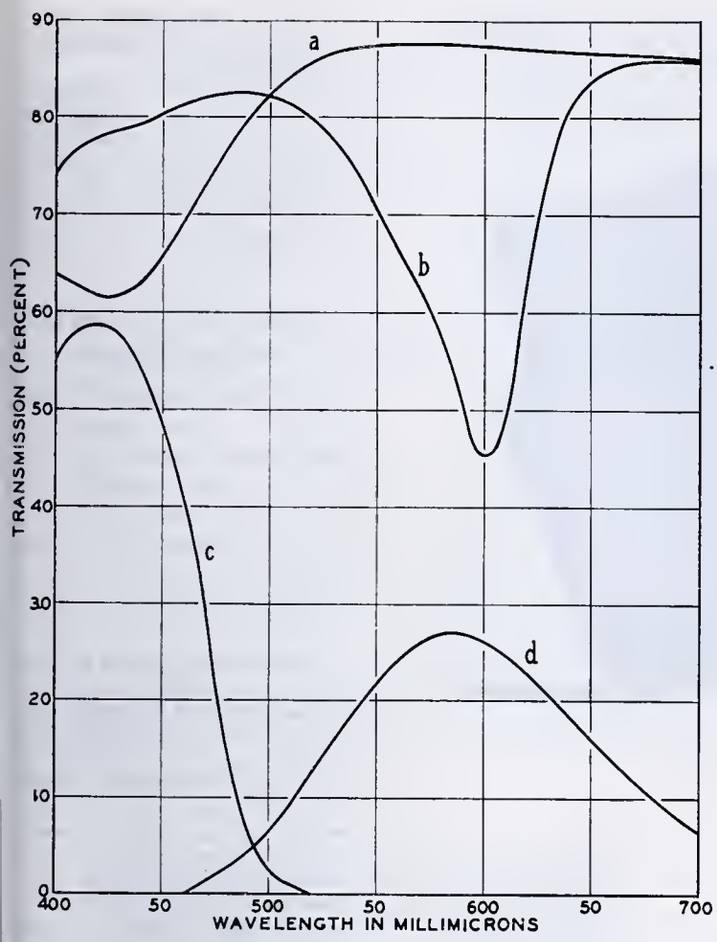

FIGURE 2. SPECTROPHOTOMETRIC CURVES OF BROMOPHENOL BLUE AND SELECTED GLASS FILTERS

a. Bromophenol blue (acid form)
b. Bromophenol blue (basic form)
c. Blue filter
d. Amber filter

In view of these facts, the authors decided to try the photoelectric method on those titrations which could not be carried out satisfactorily by visual or electrometric means.

Description and Operation of Instrument

Figure 1 (*a*) is a schematic diagram of a trial arrangement which was used successfully in these laboratories for several months. Light from a concentrated filament lamp is collimated by a lens. A circular diaphragm limits the essentially parallel beam to 2.5 cm. (1 inch) in diameter. This beam then passes through a cylindrical flask containing the solution to be titrated and impinges on a glass plate set at a 45° angle with the beam path. Approximately 8 per cent of the light passing through the flask is reflected to a barrier layer photocell in front of which is a colored glass filter. The remaining 92 per cent is transmitted by the glass plate and strikes another photocell in front of which is another colored glass filter. The two filters differ in color.

The photocells are connected with two variable resistors and a galvanometer in a Wheatstone bridge circuit originally used by Wilcox (*50*), and shown in Figure 1 (*b*). This scheme was used previously by one of the authors (*35*) in a reflectometer. Müller (*31*), in an analysis of the circuit, has derived an expression for the transmission of a solution interposed in one of the light paths for the case in which no optical filters are employed. In the present arrangement in which an optical filter of one color is used with one photocell, while a filter of a different color is used with the other photocell, the following analysis applies:

Let I = intensity of source
T_1 = transmission of sample under test for light corresponding in color to color of filter in front of photocell 1
T_2 = transmission of sample under test for light corresponding in color to color of filter in front of photocell 2
i_1 = current through resistance R_1
i_2 = current through resistance R_2
E_1 = potential across resistance R_1
E_2 = potential across resistance R_2
k_1, k_2, and k = constants depending on the characteristics of the photocells, the spectral transmissions of the colored glass filters, and the fractions of light transmitted and reflected by the 45° glass plate

Since, for low light intensity and small external resistance, the current flowing in a barrier layer photocell circuit is known to be directly proportional to the intensity of illumination,

$$i_1 = k_1IT_1 \text{ and } E_1 = k_1IT_1R_1$$
$$i_2 = k_2IT_2 \text{ and } E_2 = k_2IT_2R_2$$

At balance where the galvanometer reads zero,

$$E_1 = E_2 \text{ or } k_1IT_1R_1 = k_2IT_2R_2$$

from which

$$\frac{R_2}{R_1} = \frac{k_1}{k_2}\frac{T_1}{T_2} = k\frac{T_1}{T_2} \qquad (1)$$

$$\left(\text{where } k = \frac{k_1}{k_2}\right)$$

At the beginning of a titration, resistor R_2 is set at an arbitrary value, and R_1 is varied until balance is obtained. Then R_1 is kept fixed, and R_2 is used for balancing after each addition of titrant. From Equation 1, we see that the ratio R_2/R_1, at balance, is proportional to the ratio of the transmission of the solution for light corresponding in color to the color of the filter in front of photocell 1 to the transmission of the solution for light corresponding in color to the color of the filter in front of photocell 2. The filters are generally selected so that one transmits light in a region of the spectrum where the transmission of the solution with indicator does not change appreciably upon addition of titrant, while the other filter transmits in that region of the spectrum where the transmission of the solution with indicator changes most rapidly upon addition of titrant.

It is immaterial whether the filters are interchanged in the two light paths. This is true because a ratio of the response of the photocell having maximum sensitivity at the absorption maximum of the solution to the response of the photocell having maximum sensitivity in the region where no absorption occurs is obtained at each reading. If the two filters are reversed, a reciprocal of this ratio is obtained. This makes no difference for comparative measurements.

In some cases indicators go through their end points with a decrease in transmission in one portion of the spectrum and an increase in another. In such cases filters are chosen to transmit in these spectral regions and sensitivity is considerably increased. An example of such a case is shown in Figure 2 where spectral transmission curves of clear aqueous solutions of bromophenol blue are plotted. *a* is a spectral transmission curve of an acid solution, *b* that of a basic solution, while *c* and *d* are the transmission curves of the two filters which are used in the photoelectric photometer for titrations with this indicator.

After the feasibility of the photoelectric method had been demonstrated, an attempt was made to design an apparatus based on the same principles as that shown in Figure 1, but capable of being easily manipulated by the average technician, and rugged enough to withstand hard daily use. Figures 3 and 4 show front and rear views of the instrument which was finally constructed. Figure 5 is a circuit diagram of this instrument.

It will be observed that a third photocell is provided. It is mounted on the back of the sample compartment with its axis at right angles to the direction of the beam. When in use, it is connected in the bridge circuit with one of the photocells which receives the beam transmitted by the sample. In this manner, titrations may be carried out in which the end point is indicated by the formation or disappearance of a precipitate, or by means of a fluorescent indicator. The effect on the photocell due to light scattering is enhanced by painting the interior of the sample compartment white. An increase of turbidity or fluorescence of the solution results in an increase in the response of the cell, while the response of the cell which receives the transmitted beam decreases. Since a quantity proportional to the ratio of the two responses is measured, great sensitivity is obtained.

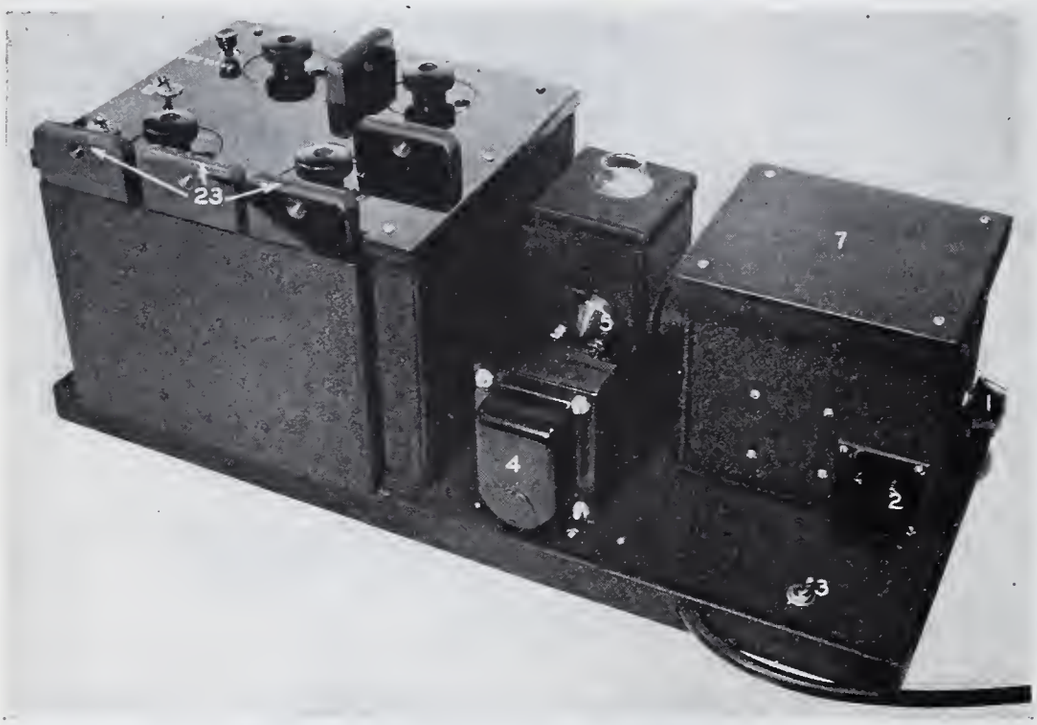

FIGURE 3. PHOTOELECTRIC TITRATION APPARATUS

1. Lamp rheostat
2. Storage battery binding posts
3. A.C.-D.C. switch
4. Step-down transformer, 115-6.3 volts
5. Fluorescence or turbidity photocell
23. Extra filters

Furthermore, the readings are practically independent of small color changes. A switching arrangement, shown in Figure 5, permits the interchanging of the three photocells in the circuit.

The power supply to the lamp of the photometer may be either direct current from a storage battery or alternating current from the 115-volt line stabilized by a voltage regulator and stepped down to 6 volts.

Model F-2 Electrocell barrier layer photocells in stainless steel hermetically sealed cases with glass windows are used. This type of construction protects the photocell element from deterioration due to corroding fumes.

Application to Dark-Colored Resins

The visual detection of the end point in the titration of dark-colored solutions by the usual indicator methods is, in most cases, uncertain. A number of attempts to overcome this uncertainty have been made.

These have included the use of a dry external indicator (*11*), the use of mixed indicators (*6*), and titration of dilute solutions in a thin cell (*27*). Coburn (*5*) employed a two-phase system where the end point was detected in the clear aqueous phase. Smith (*43*) and Tingle (*49*) used a hand spectroscope to detect the appearance of the absorption band of phenolphthalein at the end point. Pall (*36*) suggested a color-matching scheme employing a visual comparator, while Fratis and Condit (*8*) designed an Erlenmeyer flask with a side tube of small diameter through which the indicator change at the end point was more readily observed. None of the foregoing methods has been entirely satisfactory.

The apparatus described above was applied in this laboratory to the titration of dark-colored rosins and Belro and Vinsol resins.

Preliminary experiments indicated that 0.1 to 0.2 per cent alcoholic solutions of the various grades of resin which were studied were satisfactory. Since the color of such solutions is

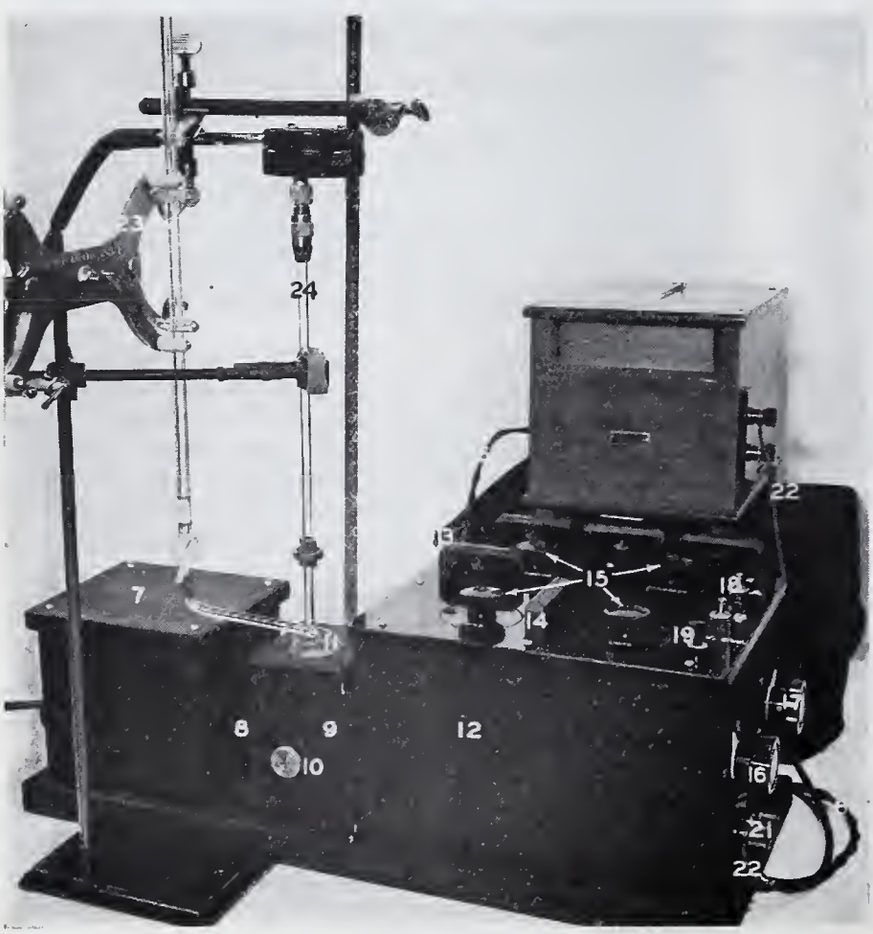

FIGURE 4. PHOTOELECTRIC TITRATION APPARATUS

6. Galvanometer lampcord
7. Lamp housing
8. Lens housing
9. Sample compartment
10. Clamp
11. Sample flask
12. Main compartment
13. Filter holder (reflected beam)
14. Filter holder (direct beam)
15. Dial resistors (Rubicon type B)
16. Rheostat for coarse adjustment (General Radio type 214A, 1000 ohm)
17. Rheostat for fine adjustment (General Radio type 214A, 100 ohm)
18. Switches for interchanging photocells
19. Locking push button switch (galvanometer circuit)
20. Galvanometer (Rubicon 3402H)
21. Binding posts for galvanometer leads
22. Galvanometer leads
23. Microburet
24. Stirrer

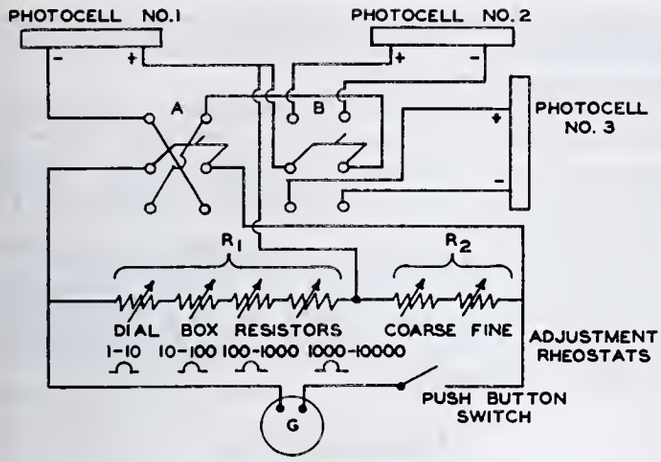

FIGURE 5. CIRCUIT DIAGRAM

orange, experiments showed that phenolphthalein with a color change of colorless to red was not a suitable indicator. Thymol blue with a change of yellow to blue was found to be very satisfactory. This indicator was suggested in 1920 by Holde (*25*) for use in the titration of dark oils and soaps. The following is a detailed description of the method that was developed for acid number determinations.

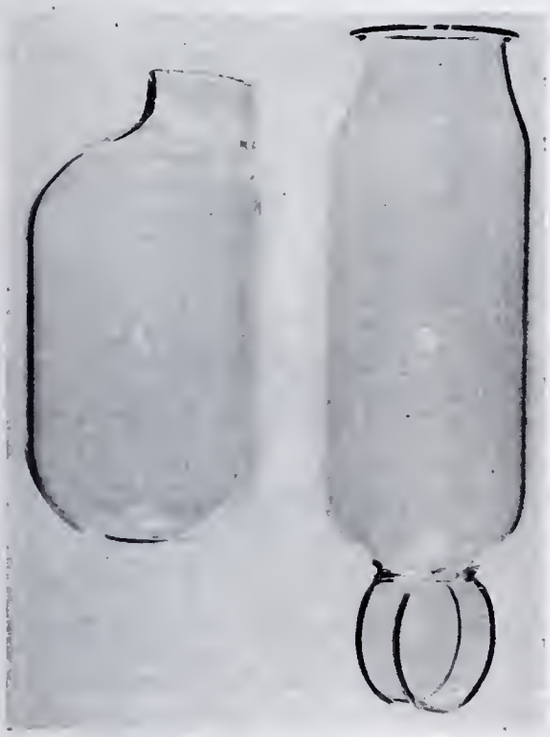

FIGURE 6. TITRATION CELLS

A. For solutions of moderate color or turbidity
B. For extremely dark or turbid solutions

Approximately 0.5 to 1.0 gram of the resin was accurately weighed out, dissolved in 95 per cent alcohol, and made up to 500 ml. in a volumetric flask. Aliquots of 150 ml. were then pipetted into a titration cell, 6 drops of 1 per cent aqueous thymol blue were added, the light was turned on, and the stirrer was started. Figure 6 shows the two types of titration cells used, *A* for solutions of moderate color and *B* for extremely dark or turbid solutions. When thymol blue is used as the indicator the filters are amber and blue, the amber being made up of Corning filters Nos. 330 (4.4 mm. thick) and 397 (2.8 mm. thick). These thicknesses are such that the maximum of the transmission of the combination occurs at 605 millimicrons for the particular melts of glass supplied. This coincides with the peak of the absorption band of alkaline thymol blue. The blue filter is a 1-mm. thickness of Corning No. 511.

Since the color change at the end point is from yellow to blue, the relative current in the arm of the bridge containing the amber filtered photocell will decrease, necessitating insertion of greater resistance for balance. Therefore, the instrument is balanced with a low resistance—e. g., 40 ohms—in the amber filtered photocell arm by adjustment of the resistance in the blue filtered arm. This latter resistance is not changed during the course of the titration. Tenth normal alcoholic potassium hydroxide is then added in small increments from a 10-ml. microburet. After each addition of titrant the resistance in the amber filtered photocell arm necessary for balance is recorded. In the neighborhood of the end point, the increments of titrant are made smaller. The titration is continued until a very marked change in color is observed.

The logarithm of the resistance in the amber filtered photocell arm is then plotted against the volume of titrant. A curve consisting of two branches at nearly right angles to each other is obtained which may be extrapolated, as in a conductimetric titration, to give an end point. A typical curve, together with a blank on the solvent, is shown in Figure 7. Two points should be emphasized: (1) It is necessary to run a blank on the alcohol. (2) A relatively large volume of indicator must be used in order to get a sufficient number of points for accurate plotting on the vertical portion of the curve.

Saponification numbers were run on several resins using 0.1500-gram samples and 10 ml. of 0.1 *N* alcoholic potassium hydroxide. The back-titration was made with 0.1 *N* alcoholic hydrochloric acid. The end points were as sharp as in the case of acid number determinations, but difficulties were found due to reaction of the alkali with the glass flasks used for saponification, and due to the readiness with which atmospheric carbon dioxide dissolves in the alcohol used for washing and transferring. These difficulties were overcome by saponifying in a silver flask, using neutral alcohol for transferring and diluting, and conducting the titration with the solution blanketed under nitrogen. Table I gives the results obtained for acid numbers and saponification numbers on samples which had been analyzed previously by electrometric titration.

The starred values in Table I were obtained by titrating the samples with alcoholic hydrochloric acid to the thymol blue end

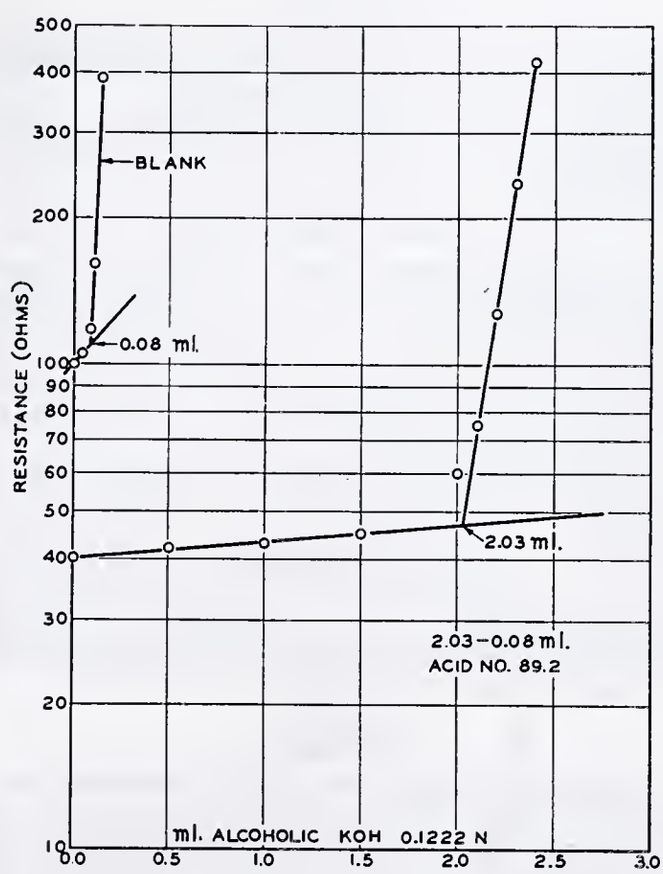

FIGURE 7. TYPICAL TITRATION CURVES

For 0.150 gram of Vinsol sample and solvent blank

Table I. Acid and Saponification Numbers

Sample	Acid No. by Photoelectric Photometer	Acid No. by Electrometric Titration
N wood rosin	163.8, 163.8, 164.7	165.0
K gum rosin	163.8, 165.0	162.9
D gum rosin	159.2, 159.8	159.8
Belro No. 1	112.7, 111.7	110.8
Belro No. 2	115.2, 113.7	114.0
Vinsol No. 1	89.2, 90.6, 87.9	88.3
Vinsol No. 2	89.4, 90.2	89.4
Vinsol No. 3	86.8, 85.8, 88.7	88.0
Vinsol No. 4	88.3, 90.7	89.8
Dehydroabietic acid	186.0, 186.0	186.0 (theory 186.9)
	Saponification No.	
N wood rosin	173.2, 173.8	172.1, 172.9
N wood rosin*	173.5, 174.5	172.1, 172.9
Belro No. 1	144.7, 145.7	139.0, 142.5
Belro No. 1*	140.0, 141.8	139.0, 142.5
Vinsol No. 1*	136.5, 137.2	135.0

point, adding bromophenol blue, and, after resetting the instrument, titrating to the bromophenol blue end point. The difference between these end points minus the corresponding difference for a blank gives a direct measure of the saponification value, since the thymol blue end point corresponds to the neutralization of the excess alkali and the bromophenol blue end point to the neutralization of the soap. The authors have found that saponifications can be made in glass flasks when this technique is used, and that the effect of atmospheric carbon dioxide is lessened. Such titrations, however, should be made under nitrogen.

Examination of data in Table I shows that the agreement between the two methods is good for a wide range of materials and that checks by the colorimetric method generally agree within ±1 per cent.

This method has been applied to research samples of very dark materials which were not entirely soluble in the solvents used. Despite the resulting turbidity and dark background color, the end point was sharp.

It is felt that this method is of use in the titration of dark-colored resins for the following reasons:

The end point may be determined with considerable certainty.
It is rapid, only 15 minutes being required for a titration and calculation.
It is simple and easily handled by a technician.
Small samples are used, 0.5 gram being sufficient for triplicate determinations. This makes it of considerable interest for research samples.

Other Applications

The foregoing technique can be applied profitably to a number of different types of reactions, and such work is now in progress in this laboratory. As in all titrations, it is necessary to pick an indicator that corresponds to the end point desired. It is extremely important to choose the proper filters, taking into consideration the spectral transmission curves of the indicator used, as well as that of the solution.

In general, this photometer appears to be adapted to all types of acid-base and oxidation-reduction titrations using colored or fluorescent indicators. Preliminary experiments indicate that it is also useful in studies of reaction rates involving changes of color, and in studies of precipitation reactions, including turbidity measurements as well as the determination of end points. By employing precision optical cells, the apparatus may be used as a sensitive chemical colorimeter.

Summary

A photoelectric photometer has been used for titrations which cannot be carried out satisfactorily by the usual visual or electrometric means. The apparatus ordinarily employs two barrier layer photocells in a balanced Wheatstone bridge circuit. In front of each photocell is a filter, one of which has a maximum transmission in the spectral region where the absorption band of the selected indicator develops, while the other has its maximum transmission in the spectral region where little or no change takes place in the indicator absorption.

The instrument has been applied successfully to the hitherto difficult determination of acid and saponification numbers of dark-colored resins. Graphically determined end points are very sharp and can be obtained in approximately 15 minutes with precisions of ±1 per cent.

The photometer is applicable to all types of titrations in which a colored or fluorescent indicator can be used. Precipitation reactions and reaction rates involving changes in solution color or turbidity can be studied. The apparatus may also be employed as a chemical colorimeter.

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An Improved Automatic Gas Effusiometer

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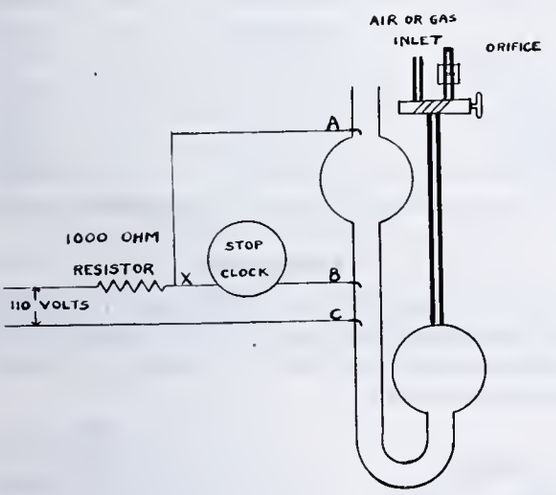
THE effusion method for the determination of the specific gravity of gases is widely used where great accuracy is not required, and is particularly adaptable to routine and control work. The method is based on the observation that the square of the time required for equal volumes of two gases under the same pressure gradient to pass through the same orifice is proportional (approximately) to their specific gravities. Thus, the specific gravity of any gas (compared with air as 1) is conveniently obtained by means of the following equation:

$$\text{Specific gravity of gas} = \left(\frac{\text{time for gas to escape}}{\text{time for air to escape}}\right)^2$$

The most widely used effusiometer is that designed by Schilling, in which a constant volume of gas or air confined over water in a glass tube is forced through a small, thin, standardized platinum orifice by the water seeking its level. The time for the water level to pass two marks on the tube is measured with a stop clock. Mitchell (*3*) among others has discussed the limitations and uses of this method.



Feskov (*1*) has described an automatic effusiometer in which he used mercury instead of water as the displacing medium and eliminated the visual observation of the moving mercury surface by employing a series-parallel electric circuit to start and stop an electric stop clock. His method eliminated the possibility of moisture condensing on the edge of the orifice, but it cannot be used with gases containing hydrogen sulfide. This limitation is very serious with most petroleum gases and led to the development of the apparatus described herein.

Improved Apparatus

This improved apparatus employs salt solution as the confining liquid and is thus particularly adapted to gases that are normally stored over brine. High-priority mercury is not required.

The apparatus consists of four parts: (1) a water-jacketed bulbed U-tube with a three-way stopcock closing one end, (2) standard orifice, (3) electric stop clock, and (4) electricr esistorsand connectors (see diagram). The glass U-tube has three platinum wire contacts fused into the open end arm of the tube. These are made of 2.5-cm. (1-inch) lengths of 20 B. & S. gage wire sealed directly into the glass. If the U-tube is made of Pyrex these seals are not gas-tight but are made water- or brine-tight by applying a thin coat of waterproof cement.

The standard orifice (obtained from The American Meter Co., Philadelphia, Penna.) may be either cemented to 3.75-cm. (1.5-inch) length of 2-mm. capillary tubing ground flat on fine emery cloth, or it may be obtained already mounted in a metal holder. The capillary tube or metal holder is joined to one arm of the capillary stopcock with rubber tubing. The orifice may be protected from the adverse effects of drafts by the modification suggested by Mitchell (*3*). The electric stop clock is a 4-watt, magnetic starter type manufactured by the Standard Electric Time Co., Springfield, Mass. The electric circuit is the series-parallel one used by Feskov, but because the salt solution between *A* and *B* acts as a resistor, only one 1000-ohm external resistor is needed. This 1000-ohm resistor may be the wire-wound, vitreous porcelain enamel type commonly used in modern radios and obtainable from radio repairmen. Some clocks may require an additional resistor, which should be placed between *A* and *X*.

The glass U-tube is filled with a salt solution to a level slightly below the contact, *B*, and the whole apparatus is lowered into a water bath, so that the external water level is slightly below the stopcock. The liquid in the U-tube may be either saturated sodium chloride solution or the acidified sodium sulfate solution suggested by Kobe and Kenton (*2*). This solution works particularly well and is prepared by adding 40 ml. of concentrated (36 *N*) sulfuric acid to 200 grams of anhydrous sodium sulfate dissolved in 800 grams of distilled water. Air or gas is introduced until all three platinum contacts are covered with brine. Because of the drain of current through *A*-*X*, there is not enough current available to allow the stop clock to run. The stop clock is then set to 0 and the stopcock turned to allow the air or gas to escape through the orifice. When the brine level falls below *A* the stop clock starts and when it passes *B* it stops because the circuit is broken. For best results the salt solution must drain freely from the walls of the tube. When the apparatus is first used, gas bubbles will appear on the platinum wires, but these will diminish until they practically disappear.

Several determinations are required to dilute and flush out the gas in the capillary tubing. When this is done, the time for the salt solution to pass from *A* to *B* is reproducible within 0.1 second, which allows specific gravities to be calculated to the third decimal place (*1*). Corrections for water vapor in the gas may be made (*3*).

Other Uses

The apparatus as described may be modified in a number of ways. The *A* contact may be made movable by supporting it with a slotted stopper. This allows a wide variation in the volume of gas forced through the orifice and a corresponding wide time range. By removing the orifice and attaching a rubber tube to the capillary tube it is possible to use the apparatus as a timing mechanism for other equipment; such a connection with a capillary viscometer tube will allow the operator to carry on other duties while the time is automatically recorded. The time of filling or emptying tanks can also be conveniently determined.

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Determination of Boiling Points of Pure Organic Liquids

A Micromethod for Use with Reduced Pressures

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AMONG the methods available for determining the boiling point of a pure organic liquid, Emich's method (*2, 3, 5*) is the most desirable in that it requires only 2 to 3 cu. mm. of material, but it can be used only at the pressure existing in the laboratory. Methods for determining the boiling point of an organic liquid under reduced pressures have been described by Smith and Menzies (*7*) and by Rosenblum (*6*), but both methods require the use of at least 50 to 100 cu. mm. of fluid. The author has observed that in organic qualitative analysis, an analyst working with small quantities prefers to use as little as possible in the determination of his physical constants, so as to have more material available for the preparation of derivatives. With this in mind, the author considered the possibility of adapting Emich's method for use under reduced pressures, and found that it could be adapted by use of a boiling point tube such as that shown in Figure 1, and using 2 to 5 cu. mm. of a pure organic liquid.

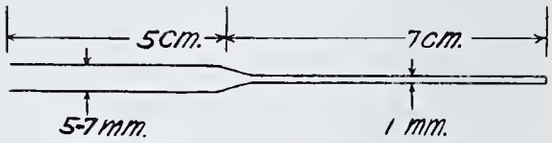

FIGURE 1

Procedure

A boiling point tube of the dimensions shown in Figure 1 is prepared from 5- or 7-mm. bore glass tubing simply by drawing out the tube into a capillary of 1-mm. bore and sealing the capillary at the end. Two to 5 cu. mm. of fluid are placed at the opening of the capillary of the boiling point tube by means of a capillary. The droplet of material is forced down to the bottom of the capillary tube by centrifuging. The boiling point tube is then connected to suction and the pressure is lowered to the desired value and determined by means of a manometer. At this point, the boiling point tube is inserted into the liquid bath (sulfuric acid was used by the author) in a Thiele melting point tube arrangement, as shown in Figure 2, and submerged 25 to 30 mm. below the surface of the liquid. The thermometer bulb should be placed as near the capillary as possible and just below the surface of the bath, but the bath liquid should cover the entire bulb. The bath is slowly heated; within close range of the boiling point condensation of material takes place in the cooler part of the capillary above the liquid level of the bath. When this occurs, the bath is heated very cautiously until a droplet which completely fills the cross section of the capillary tube starts to form (*A*, Figure 2). If the bath is heated too rapidly or heated too far above the boiling point of the liquid in the capillary tube, more than one droplet will probably form, and an incorrect result will be given. If this happens, the boiling point capillary tube should be removed and recentrifuged to bring all the fluid to the bottom of the capillary tube and the rest of the procedure repeated, heating the bath with more caution. Now the bath is allowed to cool slowly. The droplet will descend with the cooling of the bath and the boiling point is recorded as that temperature at which the droplet reaches and starts to descend below the level of the liquid in the bath.

TABLE I. BOILING POINTS OF ORGANIC LIQUIDS

Material	Hg	Boiling Point	Values in Literature (*1, 4*)
	Mm.	° *C.*	° *C*
Amyl acetate	20	46.6	47
Butyl Carbitol	24	125.9	126
Decalin[a]	22.5	81.8	81.5
	24	84	84
Hexalin[a]	21.5	72.8	73
	24	75	75
Terpineol[a]	22.5	110	(110) 114[b]
	36	120.3	(120) 125[b]
Tetralin[a]	22.5	97	97

[a] Courtesy of E. I. du Pont de Nemours & Co., Inc.
[b] The author's attention was attracted by the discrepancy between the results obtained and those given by Gardner and Brewer (*4*) in their graph of *VP vs. T*, drawn on logarithmic graph paper. Since this was the only case where such a discrepancy occurred, a close investigation of the graphs was made. The straight line of the curve did not go through three of the experimental values but when a straight line was drawn through all points, the values given above in parentheses were obtained.

TABLE II. BOILING POINTS OF ORGANIC LIQUIDS

Material	Boiling Point at 760 Mm. of Hg	Values in Literature
	° *C.*	° *C.*
Aniline	184.4	184.4
Benzene	80.0	80.1
Decalin	193.0	193.3
Hexalin	161.0	161.0
Tetralin	206	206–207.5

Discussion

The values obtained for the boiling points of several organic liquids were checked several times by the method described above and compared with those given in the literature. Typical results are tabulated in Table I.

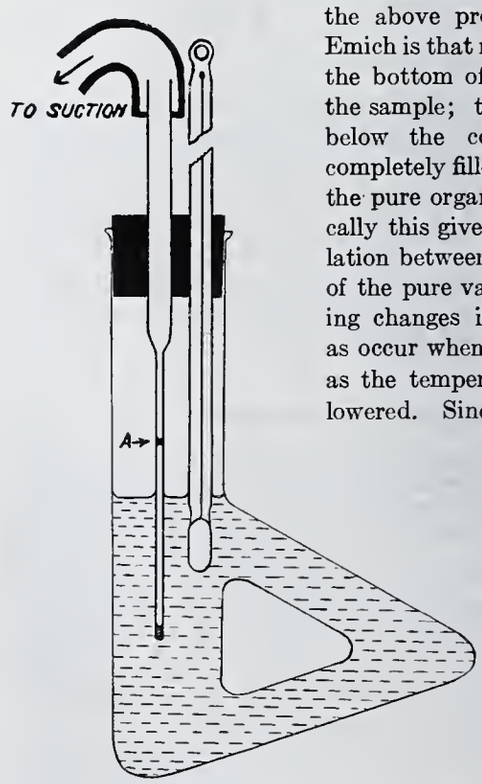

FIGURE 2

The striking difference between the above procedure and that of Emich is that no air bubble is left at the bottom of the capillary below the sample; the space which exists below the condensed droplet is completely filled with the vapors of the pure organic liquid. Theoretically this gives a more accurate relation between changes in pressure of the pure vapors and corresponding changes in temperature, such as occur when the droplet descends as the temperature of the bath is lowered. Since the boiling point is related to these effects and Emich's method does not have pure vapors below the droplet, it might be advisable to follow this procedure even in the determination of the boiling point at ordinary

conditions. Determinations by the above method at normal atmospheric conditions are reported in Table II.

Conclusions

The boiling point of a pure organic liquid using but 2 to 5 cu. mm. of material can be obtained by use of the above procedure at both reduced and normal pressures. The melting point of crystals can conveniently be determined by this method; by continuing to apply heat and at the pressure desired, the purity may be verified by determination of the boiling point.

Acknowledgment

The author is indebted to Matthew Steel, executive chairman of the Department of Biochemistry, The Long Island College of Medicine, for the laboratory facilities and encouragement which made this work possible.

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A Dithizone Method for the Rapid Determination of Copper

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A method is described for the rapid colorimetric estimation of copper with dithizone. Interference from all metallic ions except platinum, palladium, gold, silver, mercury, bismuth, and stannous tin is eliminated by extracting the copper as the dithizonate complex from an aqueous solution at pH 2.3. The reaction products of silver, gold, mercury, bismuth, and stannous tin with dithizone are destroyed by shaking the extract with acidic potassium iodide solution. Recoveries of better than ±0.3 microgram were obtained.

ESTIMATING the copper content of canned foods, tin plate, and solders requires a method capable of detecting quantities of copper as low as a few micrograms, yet the determinations must not be subject to interference by many times that quantity of other metallic ions. Various procedures have been recommended for each of these products, but no single method is well suited for all of them. Because no provision is made for the separation of copper from interfering metals in the colorimetric extraction methods of Fischer and Leopoldi (*6*) and Liebhafsky and Winslow (*9*) with dithizone, they are unsuitable in general for the analyses of the above-mentioned products. Likewise, the titrimetric extraction of copper with dithizone, a procedure described by Assaf and Hollibaugh (*1*), cannot be employed.

For a similar reason the use of organic reagents other than dithizone in procedures hitherto published is unsatisfactory. Nickel, cobalt, and bismuth interfere with the determination by means of sodium diethyldithiocarbamate, and with the recently published determination by Benzo Fast Yellow (*12*). The Biazzo method (*2*) cannot be used in presence of relatively large amounts of iron, and determinations by this method and the carbamate method are frequently complicated by the precipitation of phosphates from which copper cannot be quantitatively recovered.

Preliminary separations of copper from interfering metals by precipitation as the sulfide or by electrolysis are slow, and, in the former case, incomplete (*4*).

Several methods (*11*, *13*) have been described in which copper is separated from nickel and cobalt and from part of the bismuth by a quantitative extraction of the copper from acid solution with a carbon tetrachloride or chloroform solution of dithizone. At the same time, some of the bismuth will form the dithizonate complex and be extracted along with the copper dithizonate. The dithizonate complexes are destroyed by ashing and copper is determined colorimetrically with sodium diethyldithiocarbamate; bismuth, if present, contributes to the color and is estimated as copper.

Greenleaf (*7*) eliminated interference from bismuth by extracting the solution containing copper and bismuth dithizonates with acidified potassium iodide solution, removing bismuth as the iodide complex. The remainder of his procedure involves the oxidation of the copper dithizonate with bromine in 5 per cent sulfuric acid, and the extraction of the copper from the carbon tetrachloride layer to the aqueous layer. The aqueous solution is next digested with nitric and perchloric acids. Copper is determined on the resulting solution by the carbamate method.

From aqueous solutions having a pH value less than 3, gold, platinum, palladium, silver, mercury, bismuth, stannous tin, and copper are extracted by solutions of dithizone in chloroform or carbon tetrachloride; other metals do not react with dithizone at this pH (*5*). The first three metals mentioned may be left out of consideration because of their rarity, and only silver, mercury, bismuth, and stannous tin treated as possible interferences. Fischer and Leopoldi recommended the addition of potassium iodide solution to the weakly acidic aqueous solution containing copper and mercury with the formation of the complex ion $(HgI_4)^{--}$ as a means of eliminating interference by mercury. However, it was found in this laboratory that the amount of potassium iodide recommended by Fischer was inadequate to shield the mercury from reaction with dithizone. When larger quantities were used, the dithizone was oxidized by the iodine released in the acidic solution.

On the other hand, as the data below show, copper may be extracted by means of dithizone solution along with the other metals which react at a pH of 2.3, and then separated from these metals by shaking the extract with 2 per cent potassium iodide solution acidified with hydrochloric acid and decolorized with sodium thiosulfate. The copper remains in the carbon tetrachloride layer as the dithizonate, while the other metals are extracted as iodide complexes. The thiosulfate present in the iodide solution prevents oxidation of the dithizone by free iodine.

Experimental

Determination of Shaking Time. Since copper reacts with dithizone at a rate visibly slower than that of lead, data were accumulated showing the relationship between the time of shaking the aqueous solution of copper ions with dithizone solution and the transmittancy of the solution of copper dithizonate. The points on the curves in Figure 1 were obtained by adding 10 ml. of the dithizone solution to a 150-ml. separatory funnel containing 7 micrograms of copper in 25 ml. of 10 per cent sulfuric acid, adjusting the pH to the desired value, shaking for a given time, and then removing excess dithizone from the carbon tetrachloride layer with 25 ml. of 1 to 100 ammonium hydroxide solution. A blank of 25 ml. of 10 per cent sulfuric acid was run along with each sample. The transmittancy of the copper dithizonate solution was determined at 520 millimicrons, setting the reading of the blank in the spectrophotometer at 100 per cent transmission.

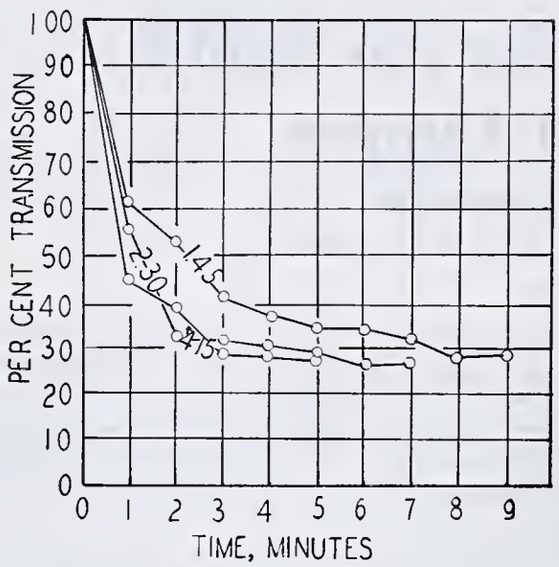

Figure 1. Extraction of Copper at Various pH Values

Figure 1 shows that at a pH of 2.3 it requires at least 6 minutes of vigorous shaking (275 cycles per minute) to obtain equilibrium between the copper in aqueous solution and the copper dithizonate in carbon tetrachloride solution when 7 micrograms of copper were originally in the aqueous solution; and that equilibrium is attained more rapidly at pH 4.15 than at the lower values. However, in practice it is advantageous to work at a pH between 2 and 3 in order to avoid extracting metals which interfere with the copper determination. Increasing the strength of the dithizone or making two extractions and combining the extracts would be expected to decrease the shaking time.

Recovery of Copper from Solutions Containing Other Metals. The desired volumes of a standard solution of copper and another metal whose effect as an interference was to be tested were run into a 150-ml. separatory funnel. The volumes were made up to 25 ml. with 10 per cent sulfuric acid. From this point the basic procedure for copper as described below was applied. The transmittancy of the dithizonate solution was determined in the spectrophotometer with the blank at 100 per cent transmission. The amount of copper in the sample was read from a calibration curve prepared with known amounts of copper. The results of experiments with mercuric, bismuth, silver, stannous, and auric ions are described in Table I.

It is evident that substantial amounts of zinc, cadmium, and bismuth do not influence the quantitative extraction of copper. Silver, mercury, and stannous tin do not interfere if the dithizone is present in a quantity more than sufficient to combine with both these ions and the copper. Zinc, cadmium, and bismuth are less readily extracted than copper and only partially accompany the copper. Silver, mercury, and stannous tin, however, are extracted more readily than copper at a pH of 2.3, so that in the presence of these elements it is absolutely necessary that the quantity of dithizone be sufficient to combine with both these elements and the copper. As dithizone complexes with silver and mercury are yellow, it is usually not difficult to detect interference from these two elements. Interference from stannous tin may be eliminated entirely by oxidizing it to the stannic state.

Interference from somewhat larger amounts of silver and mercury than are shown in the Table I could undoubtedly be eliminated by using a more concentrated solution of dithizone or by increasing the number of extractions.

Removal of Excess Dithizone by Dilute Ammonia Wash. In the removal of excess dithizone from carbon tetrachloride solution by means of an ammonia wash, the dithizone is not quantitatively removed, but an equilibrium is reached. In order to determine the effect of the concentration of ammonia in the wash solution on the transmittancy of the carbon tetrachloride layer containing copper dithizonate, in a series of experiments the concentration of ammonia was varied from 1 part in 200 of redistilled water to 5 parts in 100. Aliquots of standard copper solution were analyzed according to the usual procedure up to the removal of excess dithizone. Then each sample was shaken for 2 minutes with 25 ml. of wash solution, and the transmittancy was read against a blank. Another group was shaken for 2 minutes with 15 ml. of wash solution and then for another 2-minute period with 10 ml.

Method

Apparatus. The only special apparatus used was a Coleman spectrophotometer. Readings were made on this instrument at

Table I. Recovery of Copper

Cu Added *Micrograms*	Other Metal Added *Micrograms*	Cu Found *Micrograms*	Error *Micrograms*
	Hg^{++}		
2.5	10.0	2.7	+0.2
2.5	10.0	2.6	+0.1
5.0	10.0	5.0	0.0
5.0	10.0	5.1	+0.1
7.5	10.0	7.5	0.0
7.5	10.0	7.5	0.0
2.0	20.0	2.1	+0.1
5.0	20.0	4.9	−0.1
5.0	30.0	5.1	+0.1
2.0	40.0	1.2	−0.8
5.0	40.0	3.2	−1.8
	Bi^{+++}		
2.5	20.0	2.7	+0.2
2.5	20.0	2.7	+0.2
5.0	20.0	5.2	+0.2
7.5	20.0	7.3	−0.2
2.0	100.0	2.1	+0.1
5.0	100.0	4.9	−0.1
2.0	200.0	2.1	+0.1
5.0	200.0	4.9	−0.1
	Ag^{+}		
2.5	16.0	2.5	0.0
2.5	16.0	2.8	+0.3
5.0	16.0	4.8	−0.2
5.0	16.0	4.8	−0.2
7.5	16.0	7.3	−0.2
2.0	20.0	2.1	+0.1
5.0	20.0	4.9	−0.1
2.0	40.0	1.3	−0.7
5.0	40.0	3.2	−1.8
	Sn^{++}		
2.0	10.0	2.2	+0.2
2.0	10.0	2.2	+0.2
5.0	10.0	5.1	+0.1
7.0	10.0	6.9	−0.1
2.0	15.0	2.0	0.0
5.0	15.0	4.9	−0.1
2.0	20.0	0.0	−2.0
	Au^{+++}		
2.0	10.0	2.0	0.0
5.0	10.0	5.0	0.0
	Cd^{++}		
2.0	100.0	2.0	0.0
5.0	100.0	5.1	+0.1
2.0	200.0	1.9	−0.1
5.0	200.0	5.1	+0.1
	Zn^{++}		
2.0	100.0	2.0	0.0
5.0	100.0	5.1	+0.1
2.0	200.0	1.9	−0.1
5.0	200.0	5.0	0.0

Table II. Removal of Excess Dithizone

NH_4OH Wash	Transmittancy %
A. One shaking period	
1:200	42.5
1:200	41.0
1:100	43.0
1:100	43.0
2:100	45.0
2:100	44.0
5:100	54.0
5:100	53.0
B. Two shaking periods	
1:200	40.5
1:200	41.5
1:100	44.0
1:100	44.0
2:100	48.0
2:100	51.0
5:100	60.5
5:100	59.0

520 millimicrons, since a minimum transmittancy occurred at this wave length. A mechanical shaker is very desirable.

Reagents. Water redistilled from Pyrex was used for all dilutions. c. p. nitric acid was redistilled from Pyrex. All solutions were stored in Pyrex containers.

Glassware was cleaned with concentrated nitric acid and rinsed first with tap water, then with water redistilled from Pyrex.

Copper standard, 1 microgram of copper per ml. Accurately weigh a 0.5000-gram sample of electrolytic sheet copper and treat with 20 ml. of 6 *N* nitric acid. Evaporate the solution almost to dryness and then add 2 to 3 drops of glacial acetic acid. Transfer the solution quantitatively to a 500-ml. volumetric flask. From this stock solution a working standard of 1 microgram of copper per ml. should be prepared immediately before use.

Sulfuric acid, 10 per cent. Dilute 1 volume of the concentrated c. p. acid to 10 volumes.

Cresol red, 0.02 gram per 100 ml. of water.

Buffer solution, pH 2.3. Dissolve 38 grams of c. p. citric acid and 21 grams of c. p. disodium hydrogen phosphate dodecahydrate in redistilled water. Purify by shaking with a concentrated solution of dithizone in carbon tetrachloride. Wash out the excess dithizone with clear carbon tetrachloride, discard the washings, and dilute the aqueous layer with redistilled water to 250 ml.

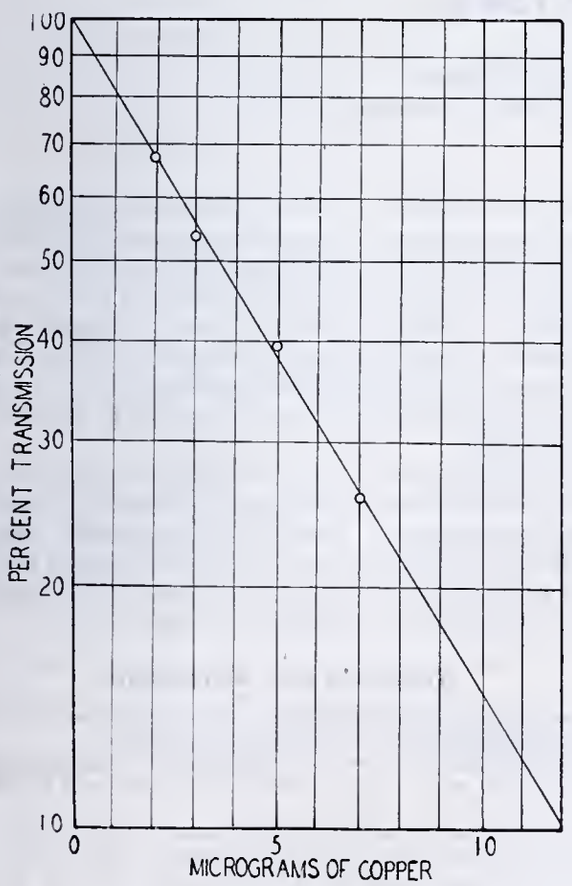

Figure Calibration Curves

When 2 ml. of this solution are added to 25 ml. of unbuffered solution, the solution is buffered at a pH of 2.3.

Dithizone solution. Dissolve 15 mg. of pure dithizone in 1 liter of c. p. carbon tetrachloride. Since solutions of dithizone are exceedingly unstable, certain precautions must be observed in their preservation (*3*). In addition, Greenleaf (*8*) has suggested the use of hydroxylamine in the sample solution itself to prevent oxidation of the dithizone.

Potassium iodide solution, 2 per cent. Dissolve 10 grams of c. p. potassium iodide in 450 ml. of water. Acidify with 5 ml. of 1 *N* hydrochloric acid. Discharge the color caused by the presence of free iodine with 0.1 *N* sodium thiosulfate solution added dropwise. Shake in a separatory funnel with successive 10-ml. portions of dithizone solution until no discoloration of the dithizone occurs. Discard the carbon tetrachloride extract and wash the aqueous layer with clear carbon tetrachloride. Dilute the aqueous solution to 500 ml. with redistilled water. Check the potassium iodide solution frequently (once a day) with dithizone solution for the presence of free iodine, and if necessary add more sodium thiosulfate solution. Since the potassium iodide solution may not be checked often enough to detect traces of free iodine, Greenleaf (*8*) suggests adding some reducing agent such as hypophosphorous acid or sodium hypophosphite to the iodide solution.

Ammonium hydroxide, 1 to 200. Dilute 1 volume of concentrated c. p. ammonium hydroxide to 200 volumes with redistilled water.

Procedure. Pipet a suitable aliquot of the sample solution into a 150-ml. separatory funnel. If the volume is less than 25 ml., make it up to that volume with 10 per cent sulfuric acid. Add 2 drops of cresol red solution and bring the contents of the flask to the yellow color of the indicator range with concentrated ammonium hydroxide. Add 2 ml. of the buffer solution (pH 2.3) and exactly 10 ml. of dithizone solution. Shake for 10 minutes. Transfer the carbon tetrachloride layer to a clean separatory funnel and add 10 ml. of acidic potassium iodide solution. Shake for 2 minutes. Transfer the carbon tetrachloride layer to another separatory funnel and add 25 ml. of 1 to 200 ammonium hydroxide solution. Shake for 2 minutes. Determine the transmittancy in the spectrophotometer at 520 millimicrons with the blank at 100 per cent transmission. From a calibration curve, such as shown in Figure 2, estimate the amount of copper present.

Table III. Analyses of Foods

Food Product	Copper Content: Dithizone method[a]	Copper Content: Greenleaf method	Copper Added	Copper Found, Dithizone Method
	P. p. m.	*P. p. m.*	*P. p. m.*	*P. p. m.*
Tomato puree	9.5	...	...	...
	8.5	...	...	...
Corn	1.03	...	1.00	2.15
	0.85	...	1.00	1.85
	0.89	...	1.00	1.95
	0.46	...	...	...
	0.84	...	...	...
	1.08	...	...	...
Pork and egg	4.5	...	...	...
Noodles	5.4	...	...	...
Pineapple	31.9	29.9	...	...
	60.9	59.6	...	...
Tomato juice	2.30	2.45	1.00	3.40
	1.80	1.70	1.00	2.90
	1.10	0.95	1.00	2.15
	2.30	2.35	1.00	3.15

[a] Values obtained from separate samples; not duplicates.

Application of Method

Food. The sample is prepared for analysis by comminution in a Waring Blendor, and then wet-ashing a suitable sample (20 to 50 grams) with concentrated sulfuric and nitric acids. The well-mixed sample is weighed into a 500-ml. Erlenmeyer flask and heated until excess moisture has been driven off and the material has just begun to char. Then 10 ml. of concentrated sulfuric acid are added to the cooled contents of the flask, and the flask is returned to the burner. Heating is continued and, when white sulfur trioxide fumes appear, nitric acid is added dropwise. As the nitric acid is consumed, more is added dropwise until the contents of the flask are colorless or at most a straw yellow in color. The flask is removed from the flame and allowed to cool. The contents are diluted with redistilled water to approximately 50 ml. Heating is resumed until sulfur trioxide fumes appear. The cooling, dilution, and heating are repeated to ensure removal of all oxides of nitrogen. The contents of the flask are transferred to a volumetric flask and diluted to volume.

Table IV. Analyses of Steel

Method	Sample 1	2	3	4	5	6
	%	%	%	%	%	%
Electrolytic (analyst 1)	0.018	0.060	0.070	0.100	0.076	0.108
Spectrographic (analyst 1)	0.020	0.053	0.073	0.092	0.073	0.122
Iodine titration (analyst 2)	0.022	0.048	0.071	0.089	0.069	0.115
Pyridine – KCNS (analyst 3)	0.020	0.056	0.074	0.094	0.070	0.108
Unknown (analyst 4)	0.026	0.062	0.067	0.100	0.075	0.103
Pyridine – KCNS (analyst 5)	0.017	0.052	0.075	0.108	0.078	0.119
Dithizone	0.018	0.058	0.083	0.103	0.075	0.115
	0.018	0.060	0.088	0.108	0.078	0.120

Samples of numerous foods were analyzed for copper. Recoveries of added copper were made from some samples, and in a few cases results were checked by the Greenleaf method (*7*). Results of these analyses are given in Table III. It is evident that the agreement between the dithizone method and the Greenleaf method is good and that copper recoveries from foods by the dithizone method are satisfactory.

Steel. Since samples of steel are analyzed occasionally for copper in this laboratory, the dithizone method was applied to a set of six steel samples which the Weirton Steel Company had distributed previously for a collaborative study of the determination of copper in tin plate base metal.

The sample was prepared for analysis by dissolving 1 gram of steel filings in 100 ml. of hot 1 to 9 sulfuric acid. Redistilled nitric acid was added dropwise and heating continued until the solution was a clear yellow color. After cooling it was diluted to 250 ml. in a volumetric flask. A suitable aliquot was then analyzed for copper.

In Table IV data obtained by the dithizone method are to be found, together with data furnished by Weirton Steel Company on the results of the collaborative study. It is apparent that the dithizone method may be applied to steel without modification.

Solder. A 1.0-gram sample was dissolved in concentrated hydrochloric acid and bromine. After filtering off the lead chloride the solution was neutralized to a pH of 2 to 3. Copper was determined in a suitable aliquot by the regular procedure. The copper content of Bureau of Standards Sample No. 127 was found to be 0.016 per cent as compared with 0.013 per cent determined spectrochemically and 0.014 per cent chemically by Ruehle and Jaycox (*10*).

Acknowledgment

The authors wish to thank G. C. Jenison of the Weirton Steel Company for supplying steel samples and the results given in Table IV.

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Determination of Tungsten in Low-Grade Tungsten Ores

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Two methods based on the Feigl-Krumholz test are described for rapid colorimetric determination of small amounts of tungsten in low-grade ores and tailings. Aliquot portions are adjusted to deal with quantities of tungsten ranging from 0.04 to 0.40 mg. of WO_3. The maximum permissible concentrations of possible interfering ions have been determined and a graphical method of correcting for the usually slight interference of molybdenum is given.

THE time required for a tungsten analysis by gravimetric methods is a disadvantage in exploring for tungsten deposits or in control work on mill tailings. Some uncertainties are introduced in the determination of very small amounts of tungsten because of the possible interference of high concentrations of foreign ions introduced by the large sample necessary for the analysis.

Procedures for the rapid determination of small amounts of tungsten have been reported by Russian chemists, but the literature is available to American laboratories only through abstracts. In view of the strategic importance of tungsten, the writers thought it desirable to report some new results on methods for its estimation based on the Feigl-Krumholz test.

Feigl and Krumholz (*1*) showed that a weakly alkaline tungstate solution would slowly develop a yellow color upon the addition of thiocyanate and an acid stannous chloride solution. The color reached a maximum after 30 minutes and it was found that Beer's law was valid from 0.01 to 0.1 mg. of tungsten trioxide. Fernjančič (*2*) substituted titanous chloride for stannous chloride and developed the same color instantaneously. Other investigations along similar lines have been carried out by Russian chemists (*3, 5, 6, 8, 9*).

In applying the test to ores the authors preferred to retain stannous chloride as the reducing agent because it is colorless and less care is necessary to obtain satisfactory results. Stannous chloride is preferable when much iron is to be reduced and necessary in the presence of phosphate, as certain combinations of titanium and phosphorus also produce a yellow color.

Reagents and Apparatus

Potassium thiocyanate solution. Dissolve 25 grams of reagent grade potassium thiocyanate in 100 ml. of water.

Stannous chloride solution. Dissolve 20 grams of the dihydrate in 100 ml. of concentrated hydrochloric acid and 100 ml. of water.

Sodium hydroxide solution 1 (approximately 40 per cent). Dissolve 40 grams of sodium hydroxide in 60 ml. of water.

Solution 2 (approximately 20 per cent). Dissolve 20 grams of sodium hydroxide in 80 ml. of water.

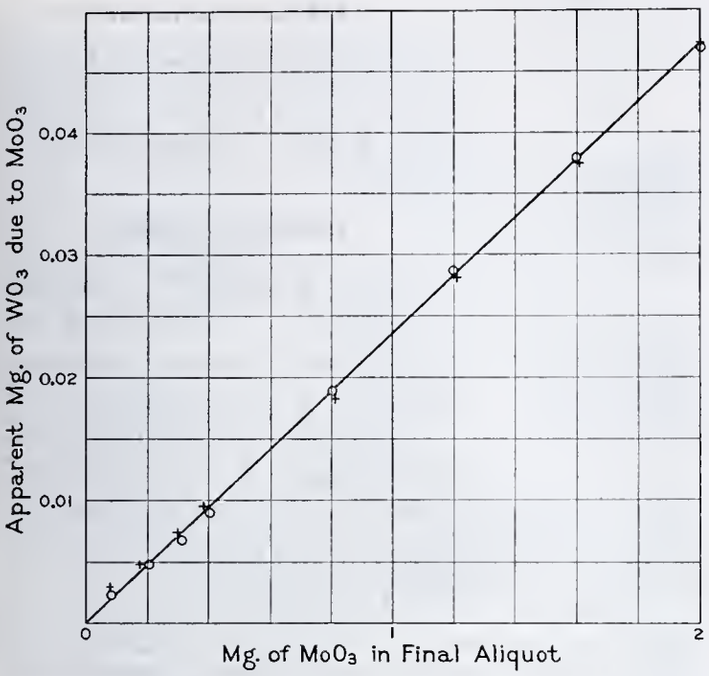

FIGURE 1. INTERFERENCE OF MOLYBDENUM

Standard tungstate solution. Solution 1. 1 ml. = 0.002 gram of WO_3. Dissolve about 0.71 gram of molybdenum-free sodium tungstate dihydrate in water and dilute to 200 ml. in a volumetric flask. Check the tungstic oxide content by gravimetrically determining WO_3.

Solution 2. 1 ml. = 0.00004 gram of WO_3. Dilute a suitable volume of solution 1.

Colorimeter. A Klett colorimeter was used in this work.

Experimental

For convenience, the Feigl-Krumholz procedure was modified by using a larger volume for final comparison. A lower concentration of stannous chloride than originally recommended was found to give satisfactory results. The final volume of the test solution used by the writers was 26.5 ml.

One milliliter of potassium thiocyanate reagent was added to 10.5 ml. of the experimental tungstate solution containing about 0.12 gram of free sodium hydroxide. This corresponds to an alkalinity of about 0.3 *N* and was used for all subsequent tests. Next, 5 ml. of stannous chloride solution and 10 ml. of concentrated hydrochloric acid were added. The color was compared in a colorimeter with a similarly treated standard.

Under these conditions Beer's law was valid up to 0.5 mg. of tungsten trioxide.

Iron, antimony, calcium, magnesium, aluminum, phosphate, sulfate, combinations of iron and phosphate, iron and sulfate, ammonium, and sodium salts do not interfere. Soluble silica does not interfere but may occasionally precipitate out. Iron accelerates the development of the tungsten color.

Among the colored metal ions tested, those listed in Table I do not interfere if the final aliquot contains less than the quantities indicated. A few of these elements interfere still less if the tungsten color is concentrated by means of an organic solvent, such as amyl acetate.

There is no interference when phosphates are present with smaller quantities of vanadium than indicated in Table I. Chromates have not been encountered in tungsten ores. Copper precipitates out as cuprous thiocyanate. If sufficient thiocyanate solution is present, however, the supernatant liquid will give the characteristic test for tungsten. By experiment, 0.1 gram of copper required 2 ml. of potassium thiocyanate solution for satisfactory development of the tungsten color. Lead precipitates out as the chloride when over 60 mg. of lead are present, but no interference with the development of the color was noticed. Citric acid, in amounts in excess of 0.5 gram, interfered with the maximum development of the color. Of course nitrates and other oxidizing agents interfere with the reducing action of stannous chloride. More than 1 gram of sodium chloride or 2 grams of ammonium chloride will precipitate from the final acid solution but do not alter the tungsten color. Arsenic should be absent; it precipitates out slowly as metallic arsenic. The interference ranges from a brownish opalescence to a black precipitate according to the amount of arsenic present. This behavior of arsenic is very characteristic and easily recognized. Titanium in quantities under 10 mg. does not interfere appreciably. Larger quantities produce a yellow color in the solution which is intensified in the presence of phosphate.

TABLE I. LIMIT OF INTERFERENCE OF CERTAIN ELEMENTS

Element	Maximum Amount Permissible in Aqueous Solution	Maximum Amount Permissible with Extraction
	Mg.	*Mg.*
Ni	3. Ni	No interference
Co	1. Co	20 Co
Cr	0.3 Cr	0.4 Cr
V	0.2 V	1.3 V

Molybdenum produces a weak yellow color similar to the yellow of tungsten. The interference of molybdenum is illustrated in Figure 1. The data for the curve in Figure 1 were obtained by applying the general method to various mixtures of tungstate and molybdate. The interference of molybdenum is negligible when present in the final aliquot in quantities below 0.6 mg. of molybdenum trioxide. For rapid work the curve may be used to apply corrections. Molybdenum itself may be quickly determined colorimetrically in a separate aliquot of the same solution used for tungsten (*4*).

Procedures for Tungsten Ores

1. GENERAL METHOD. Fuse 1.0 gram of sample with 5 grams of sodium peroxide in an iron crucible. Digest the cooled melt with hot water containing a little alcohol to assist in reducing manganate. After the peroxide is destroyed filter into a 250-ml. volumetric flask, washing the residue well with warm 0.5 per cent sodium hydroxide solution. To a 10-ml. aliquot in a 50-ml. beaker add by pipet 0.5 ml. of water, 1 ml. of potassium thiocyanate solution, 5 ml. of stannous chloride solution, and 10 ml. of concentrated hydrochloric acid in the order mentioned. A standard containing 0.20 mg. of WO_3 should be prepared at the same time. For this purpose pipet into a 50-ml. beaker 5 ml. of standard tungstate solution, 5 ml. of water, and 0.5 ml. of sodium hydroxide, Solution 2. Follow with the reagents mentioned above. After 1.25 hours match the standard against the sample in a colorimeter. Best results are obtained when the standard and the sample have about the same tungsten concentration.

2. PREFERRED METHOD WHEN MOLYBDENITE OR ARSENIC IS PRESENT. During the solution of the sample in this method, and in the absence of oxidizing agents, molybdenite is not attacked and arsenic, volatilizing as arsenic trichloride, is effectively removed. This method is adapted to the determination of small quantities of tungsten because alkali salts are kept to a minimum and no collection methods need be used for concentrating the tungsten.

When acid-soluble titanium minerals are present in excess of 1 per cent titanium dioxide, this method tends to give low results because of some tungsten retention by titanium phosphate. The writers' experience with tungsten ores from many localities indicates that soluble titania generally does not exceed 0.2 per cent titanium dioxide, a quantity which does not interfere.

To 0.5 to 2 grams of finely ground sample in a 100-ml. beaker, add 1 ml. of 1 to 3 phosphoric acid and 40 ml. of concentrated hydrochloric acid. Cover the beaker and allow the solution to digest on the steam bath for about 20 minutes. Remove the cover and evaporate the contents to dryness. Add 10 ml. of 1 to 4 hydrochloric acid and digest for at least 10 minutes. Dilute to 50 ml. with water and warm. Add paper pulp, and filter into a 100-ml. volumetric flask. Wash the residue with water.

To a 10-ml. aliquot add 0.5 ml. of sodium hydroxide, Solution 1, and 1 ml. of potassium thiocyanate solution. Now add the stannous chloride and hydrochloric acid as in procedure 1. Allow the mixture to stand 1.5 hours and compare with a standard made at the same time in the manner indicated in procedure 1.

Table II. Colorimetric Estimation of Tungsten

No.	Mineral	Location	Per Cent of WO_3 Procedure 1, colorimetric	Procedure 2, colorimetric	Gravimetric	Notes
1	Scheelite	Alaska	...	0.08	0.04	...
2	Scheelite	California	0.14	0.15	0.10	...
3	Scheelite	Alaska	...	0.11	0.11	Contained As
4	Scheelite	Alaska	...	0.18	0.19	Contained As
5	Scheelite	Alaska	0.31	0.31	0.26	...
6	Scheelite	Alaska	0.45	0.46	0.48	...
7	Psilomelane	Arizona	0.54	0.55	0.52	Total Mn 48% BaO 14%
8	Scheelite	Alaska	0.60	0.63	0.60	...
9	Scheelite	Alaska	0.71	...	0.68	...
10	Scheelite	Alaska	0.73	0.74	0.75	...
11	Coronadite	New Mexico	2.0	...	1.85	Pb 30%
12	Scheelite	Alaska	1.1	...	1.12	...
13	Psilomelane	New Mexico	1.2	...	1.29	Total Mn 50% BaO and SrO 15%
14	Molybdenite	Idaho	...	None	None	MoS_2 1.86%

Results on Tungsten Ores

The tungsten ores were first analyzed gravimetrically, using 4-gram portions essentially by the procedure outlined by Scott (*7*). Colorimetric estimations using procedures 1 and 2 are given in Table II for comparison; these results are seen to be good in the range up to 1 per cent tungsten trioxide.

Acknowledgment

The authors wish to thank M. Fleischer for the analyses of samples 7 and 13 and C. Milton for No. 3; also William Schlecht and R. C. Wells for kindly reviewing the manuscript.

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Microbiological Determination of Pantothenic Acid

Further Studies

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The procedure for microbiological determination of pantothenic acid has been modified by additions to the basal medium and improvements in the method of growing inoculum. The modified method is capable of measuring smaller amounts of pantothenic acid and gives concordant results at increasing levels of sample.

Although published methods for the microbiological assay of pantothenic acid (*8, 13*) have been widely used and have been made the basis for various conclusions regarding the physiological and nutritional functions of this vitamin, certain discrepancies raise serious questions as to the reliability of the results. Among these difficulties may be listed lack of agreement with animal tests, the large "drift" frequently observed, and the fact that minor variations in the method of preparing the sample may cause wide fluctuations in the final assay values. Furthermore (*13*), anomalous results were obtained in attempting to carry out recovery experiments on hydrolyzed samples of fresh liver and kidney.

Some of the reasons underlying these troubles have recently come to light. The effect of fatty acids on the growth of *Lactobacillus casei* has been discovered (*2, 12*), and methods for avoiding their interference in the riboflavin assay have been worked out (*1, 12*). It has also become apparent that certain water-soluble substances of an unknown nature, and apparently present mainly in brans, are capable of stimulating the bacterial response (*5, 7, 15*).

The present paper is concerned (*a*) with a study of methods for liberating "bound" pantothenic acid in the sample, (*b*) with procedures designed to remove interfering fat-soluble substances from the test solution, and (*c*) with efforts to modify the basal medium in such a way as to avoid the effect of water-soluble, stimulating factors and to prevent "drift".

Except for the modifications detailed below, the general procedure previously described (*13*) has been followed in the present work for setting up assays and calculating results.

Cultures and Inoculum

Evidence has accumulated in carrying out a large number of assays that the best response is obtained when the inoculum is not carried through several transfers in the liquid medium as formerly recommended, but is grown directly from a stab culture. It also appears advisable to transfer the stab cultures weekly instead of monthly. To grow inoculum, cells from a suitable stab are transferred into a tube containing 10 cc. of the basal medium (see below) plus 1 microgram of calcium pantothenate. After 24 hours' incubation at 37° (±1°) C. the cells are centrifuged out and resuspended in 10 cc. of saline, and the resulting cell suspension is used as inoculum. Thus there is no "subculture" made between the stock stab culture and the inoculum and the cell suspension is not so extensively diluted with saline as before (*13*). One should return to a stab each time inoculum is to be grown. One stab culture may be used several times if proper precautions are taken to avoid bacterial contamination.

Basal Medium

The previous basal medium has been modified by adding glutamic acid and a supplement prepared from Vitab, by increasing

the sodium acetate concentration to 2 per cent, and by using a more concentrated yeast supplement. The remaining ingredients of the medium were prepared as previously described (*13*).

Yeast Supplement. This was prepared as before (*13*) except that Darco G-60 was used for the adsorption, and the adsorption was carried out in a volume of 240 ml. After the second charcoal treatment the filtrate was neutralized with solid sodium hydroxide and diluted to 250 ml. The supplement was thus four times as concentrated as previously described, since each milliliter was equivalent to 100 mg. of whole autolyzed yeast.

Vitab Supplement. Seventy-five grams of Vitab (National Oil Products Co.) were dissolved in 500 ml. of water, the pH was adjusted to 1.2 to 1.5 with concentrated hydrochloric acid, and the mixture was stirred with 50 grams of activated charcoal—e. g., Darco G-60—for 20 minutes at room temperature. The charcoal was filtered off, the pH readjusted to 1.2 to 1.5, and the adsorption repeated. The filtrate was neutralized to pH 6.8 with concentrated sodium hydroxide, filtered again, and diluted to 1000 ml. with distilled water. One milliliter of the final solution therefore contained the equivalent of 75 mg. of Vitab.

Glutamic Acid. One gram of *l*-glutamic acid hydrochloride was dissolved in 80 to 90 ml. of water by warming on the steam bath and stirring until solution was effected. The solution was then cooled and diluted to 100 ml.

The amounts of these materials used in setting up an assay, and the composition of the modified medium are shown in Table I. This medium will be referred to henceforth as the basal medium. To set up a 100-tube assay, the amounts of the various reagents and solutions indicated in Table I are mixed, adjusted to pH 6.6 to 6.8, and diluted to 500 ml. with distilled water. This gives a mixture having twice the concentration of the basal medium. The rest of the procedure is carried out as before (*13*).

Table I. Composition of Basal Medium and Amounts Required for One Hundred Tubes

Ingredient	Amount for 100 Tubes	Amount in Medium
	Ml.	%
NaOH-treated peptone	100	0.5
	Grams	
Anhydrous glucose	10	1.0
Sodium acetate	14	2.0
	Ml.	
Cystine	100	0.01
Asparagine	25	0.025
Glutamic acid	25	0.025
Riboflavin	2	0.1 p. p. m.
Yeast supplement	50	Equivalent to 50 mg. of autolyzed yeast per tube
Vitab supplement	100	Equivalent to 75 mg. of Vitab per tube
Salts solution A	5	(5 ml. of each per liter of medium)
Salts solution B	5	

Standard Curve

Since the linear portion of the standard curve extends to 0.08 to 0.10 microgram of calcium pantothenate, rather than to approximately 0.15 microgram as was formerly the case, it has seemed desirable to work with a more dilute standard solution. The standard solution now used is prepared as previously described, but is diluted to 0.02 microgram per ml. The standard curve is constructed from the titration values of tubes containing 0.00, 0.00, 0.5, 0.5, 1.0, 1.0, 2.0, 2.0, 3.0, 3.0, 4.0, 4.0, 5.0, and 5.0 ml. of the standard solution. A typical standard curve, given in Figure 1 (curve 1), shows that the useful assay range is about 0.01 to 0.08 microgram of calcium pantothenate per tube.

Preparation of Materials Tested

Dry samples are finely ground. Fresh tissues are homogenized with a small amount of water in a Waring Blendor or Potter-Elvehjem homogenizer (*9*). An amount of the material containing approximately 10 micrograms of pantothenic acid is then suspended in 100 ml. of distilled water and adjusted to pH 6.8. If recovery experiments are to be carried out, the amount of sample taken is reduced by one half, and a known amount of pantothenic acid added as the volume is being made up to 100 ml. The amount of pantothenic acid added should be approximately equal to the quantity of the vitamin in the sample. In either case the mixture is autoclaved for 15 to 20 minutes at 1 kg. per sq. cm. (15 pounds per square inch) pressure. After cooling, 2 ml. of 2.5 *M* sodium acetate are added, and the mixture is brought to pH 4.8 (green to bromocresol green, outside indicator) with *N* hydrochloric acid. The solution is then diluted to 200 ml. and is filtered through a fluted quantitative paper—e. g., Whatman No. 40. If the filtrate is not perfectly clear it is refiltered with the aid of a small amount of fine Filter-Cel. Fifty milliliters of the filtrate are neutralized to pH 6.8 with *N* sodium hydroxide, and diluted to 100 ml. for assay.

If a sample very high in fat is to be assayed, or if the sample as above prepared shows a drift, it is necessary to extract the 50 ml. of filtrate with ether after neutralizing but before diluting to volume. The filtrate is shaken out with about 30 ml. of ether. The extraction is twice repeated, and the aqueous phase is then diluted to 100 ml. without removal of the dissolved ether.

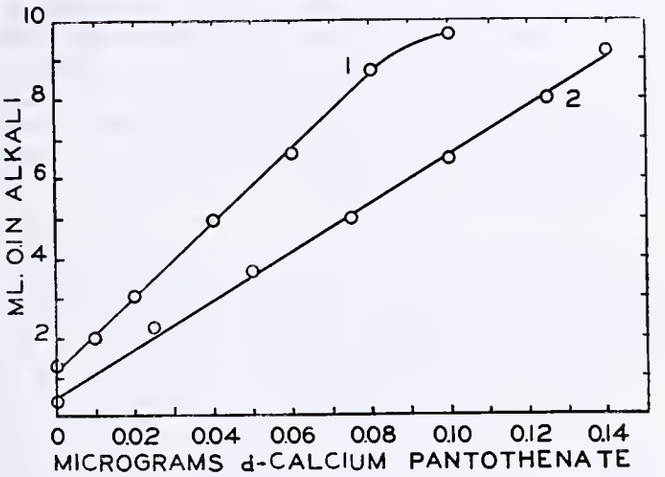

Figure 1. Standard Curves

Curve 1, present basal medium. Curve 2, original medium. 72 hours' incubation.

Enzyme Treatment. An aliquot of the homogenized sample estimated to contain 10 micrograms of pantothenic acid after digestion, is suspended in 25 ml. of distilled water plus 2 ml. of 2.5 *M* sodium acetate. An amount of clarase or papain powder equal to twice the dry weight of the sample is added and the mixture brought to pH 4.8 with *N* hydrochloric acid. The volume is made to 50 ml. with water and the mixture incubated at 37° C. under toluene for 48 hours. The suspension is then prepared for assay as described above. A portion of the enzyme powder is carried through the same procedure and assayed to ascertain its pantothenic acid content.

Digestion with pancreatin was carried out in a similar manner, except that 50 ml. of 0.05 *M* phosphate buffer, pH 7.2, replaced the sodium acetate.

Ether-Soluble Fraction from Yeast. A pancreatin-digested yeast suspension was adjusted to pH 6.8 and subjected to continuous ether extraction for 3 hours. The ether was removed from the extract, the residue taken up in 5 ml. of alcohol, and the solution diluted to the same volume as that of the aqueous phase extracted.

Oleic Acid. The oleic acid was prepared by saponifying a pure sample of ethyl oleate and was tested in the form of a fine suspension obtained by diluting a methanol solution with water. The small amount of methanol used had no detectable effect on the test organism.

Results and Discussion

Although satisfactory agreement was reported (*13*) for animal and microbiological pantothenic acid determinations on several samples, a further comparison has revealed that in the case of yeast serious discrepancies exist. Thus the data in Table II show about fivefold higher values by chick assay of a number of yeast samples than by the *L. casei* method as originally published. It has been reported (*13*) that such discrepancies are attributable to a "bound form" of pantothenic acid not measured by the bacteria, and efforts have been made to release it by enzymatic digestion (*4, 10, 14, 16*).

Table II. Comparison of Animal and Microbiological Assay of Pantothenic Acid in Yeast

Dry Yeast, Sample No.	Pantothenic Acid Found: Chick assay[a]	Microbiological Assay[b]: Direct	Microbiological Assay[b]: Clarase-digested
	Micrograms per gram		
106	200	47	134
107	220	45	187
108	190	38	123
109[c]	510	131	298
110	250	52	132

[a] Chick assays carried out by Jukes by method previously described (*6*). Each value is average of 2 or 3 concordant individual assays.
[b] Carried out according to original procedure (*13*).
[c] A fortified yeast sample.

In the early part of this work a study was made of the effectiveness of clarase, pancreatin, and papain for the liberation of bound pantothenic acid in yeast. Systematic variations in the amount and type of enzyme and in the conditions of time, temperature, and pH under which the digestions were carried out showed that the values obtained could be increased to a maximum of about 60 to 80 per cent of those found by chick assay. Some of these results are given in Table II.

However, many of the assays run in connection with this study showed considerable drift—i. e., a regular decrease in apparent pantothenic acid content was noted as larger amounts of sample were tested. The amount of drift was usually increased following the enzyme treatment and it was difficult to obtain concordant duplicate results. Typical data are shown in Table III. Since the authors' experience in general has been that assays showing drift are unreliable, it was questionable whether the increase found after enzyme treatment actually represented pantothenic acid. Inasmuch as methods of avoiding such drifts in the riboflavin assay had in the meanwhile been worked out (*12*), it seemed desirable to apply similar procedures to the digested yeast samples. When this was done, lower values for the apparent pantothenic acid content were obtained. As shown in Table III, filtration or ether extraction reduced the value found for yeast 110 after clarase treatment by about 20 per cent, but the result was still twice that obtained by assay of the original sample. When the same yeast was digested with pancreatin, and then assayed, a value of about 210 micrograms per gram was found. This value was reduced to 47 after filtration of the digest and to 41 after ether extraction. Similar treatment after papain digestion gave a value of 42 micrograms per gram. Control experiments showed that these procedures did not remove pantothenic acid. It was evident, therefore, that a large part of the increase observed after enzyme treatment was attributable to the liberation of interfering substances, rather than pantothenic acid, and it seemed likely that the interfering substances were fat-soluble in nature.

This suspicion was strengthened by testing the effect of oleic acid and an ether-soluble fraction from yeast. The data presented in Table IV summarize the results of this work. It is evident that as in the riboflavin method (*12*), oleic acid exerts a large influence on the bacterial response, and that the magnitude of the stimulation or inhibition observed is dependent not only on the amount of fatty acid used but also on the level of pantothenic acid being tested. The ether extract of yeast was likewise stimulatory.

In further experiments it became obvious, however, that even after fat-soluble interfering substances were removed, an objectionable amount of drift still persisted (Table III). It was assumed, therefore, that water-soluble materials of a stimulatory nature were being introduced with the sample, and efforts were made to prepare a supplement to the medium which would provide such stimulants in excess. Since it has been reported that water extracts of various brans contain materials of an unknown nature which stimulate growth and acid production by *L. casei* (*5, 7, 15*), a rice-bran concentrate, Vitab, was selected as the starting material. It was successfully freed of pantothenic acid by charcoal treatment.

When the basal medium was modified by the addition of this Vitab supplement together with extra sodium acetate (*3, 11*), a steeper standard curve was obtained (Figure 1), and the drift was almost entirely eliminated. The data in Table V show excellent agreement over a wide range of increasing levels of sample, even though widely different types of natural materials were analyzed. It is important in carrying out these analyses to employ wide ranges in sample levels; otherwise a moderate amount of drift may easily be overlooked. In the present work the criterion has been set up that in order for an assay to be regarded as reliable, at least three sets of duplicate tubes must fall in the assay range—i. e., contain amounts of pantothenic acid corresponding to the linear portion of the standard curve—and that the results calculated from any one of these tubes must not deviate by more than ±10 per cent from the average. Furthermore, the amount of sample in those tubes representing the highest level should be at least four times that at the lowest level.

With this improved assay procedure available it has been possible to re-examine with more confidence the question of enzyme digestion of the sample. The data in Table VI show clearly that it is often necessary to include an enzyme treatment in order to

Table III. Effect of Method of Preparation of Sample on Apparent Pantothenic Acid Content of Yeast[a]

Test Solution Added per Tube	Treatment of Sample: Suspended in water and assayed directly	Digested with Clarase: Suspension assayed directly	Digested with Clarase: Filtered	Digested with Clarase: Extracted with ether
Ml.	Micrograms per gram			
1	57	165	...	...
1	58	161	...	...
2	49	121	114	114
2	53	117	118	118
3	42	105	102	102
3	41	109	102	102
4	..	...	86	90
4	..	...	86	90
Av.	50	130	101	103

[a] Experiments done on yeast 110, and assays carried out according to original procedure (*13*).

Table IV. Effect of Fatty Materials on Recovery of Pantothenic Acid

Fatty Material Added per Tube: Type	Amount	Pantothenic Acid Added per Tube	Pantothenic Acid Recovered
	μg.	*Microgram*	%
Oleic acid	28.2	0.025	116
	28.2	0.050	100
	28.2	0.075	94
	28.2	0.100	92
	56.4	0.025	176
	56.4	0.050	156
	56.4	0.075	118
	56.4	0.100	97
	112.8	0.025	84
	112.8	0.050	183
	112.8	0.075	132
	112.8	0.100	100
	169.2	0.025	125
	169.2	0.050	192
	169.2	0.075	138
	169.2	0.100	105
	Ml.		
Ether extract of yeast[a]	1	0.100	96
	2	0.100	94
	3	0.100	94
	1	0.050	153
	2	0.050	136
	3	0.050	127
	4	0.050	131
	3	0.025	159

[a] 1 ml. contains extractable substances from 1 mg. of dried yeast obtained as described in text.

achieve maximum values. It has not yet been possible to carry out by the improved assay procedure an extended comparison of the effectiveness of various types of enzyme treatment of different samples. Whether the clarase digestion described represents the optimum method for liberating combined pantothenic acid must be left an open question at present.

Table V. Agreement at Increasing Levels of Sample

Test Solution Added per Tube	Pantothenic Acid Found				
	Fresh hog liver	Hydrolyzed hog liver[a]	Canned tomato juice	Canned peas	Oil-packed sardines
Ml.	*Micrograms per gram*				
0.5	15	1.3	0.49	0.90	3.2
1.0	17	1.6	0.45	0.90	2.8
1.5	17	1.5	0.43	...	2.7
2.0	15	1.6	0.43	1.08	2.9
2.5	17	1.5	0.42	...	3.0
3.0	16	1.5	0.42	0.96	3.0
3.5	15	1.4	...	...	2.9
4.0	16	1.4	...	0.84	3.2
Av.	16	1.5	0.44	0.96	3.0

[a] Suspended in 6.7 volumes of 2 *N* sodium hydroxide, autoclaved 1.5 hours at 15 pounds per sq. inch pressure, filtered at pH 4.8, and neutralized.

Table VI. Effect of Enzyme Treatment on Determination of Pantothenic Acid in Foods

Sample	Pantothenic Acid Found	
	Direct	Clarase-treated[a]
	Micrograms per gram	
Sardines	3.0	6.5
Pineapple	0.56	0.8
Beets	0.89	0.94
Peas	0.98	1.8
Spinach	0.42	0.55
Yeast no. 110	52	98
Yeast no. 107	54	130
Tuna fish	1.5	2.4

[a] Corrected for pantothenic acid content of enzyme preparation used.

That the enzyme does not destroy pantothenic acid, and that no appreciable amount of vitamin is lost by the filtration of the test suspension, are indicated by the recoveries given in Table VII. The recovery on clarase was made by treating 5 micrograms of calcium pantothenate with 2 grams of clarase.

The results in Table V relating to alkali-hydrolyzed fresh liver are noteworthy. It was possible to carry out an entirely satisfactory analysis on this material, and recovery of added calcium pantothenate was good. This is in sharp contrast to previous experience (*13*). The slight residual activity is not destroyed by further treatment with alkali under conditions which result in complete inactivation of pantothenic acid.

No effort has been made in the present work to extend the range of the standard curve to titration levels appreciably above 10 ml., although it is well known that if both the glucose and pantothenic acid are increased much larger amounts of lactic acid will be formed. Extending the titration range above 10 or 11 ml. is considered inadvisable, since the large amount of acid formed may unfavorably influence the growth and vigor of the test organism. Furthermore, no one has yet succeeded in devising a basal medium which will permit a continuation of the linear response to still higher titration values. By linear response is meant a constant ratio between added increments of the vitamin to be determined and the extra acidity thereby produced.

It is important that this ratio be as high as possible in order for the assay to be sensitive, accurate, and specific. For example, the titration values for the standard curve (Figure 1, curve 1) go from 2.0 to 8.7 ml. as the amount of calcium pantothenate is increased from 0.01 to 0.08 microgram. This corresponds to an average increased titration in this region of the curve of $(8.7 - 2.0) \div (0.08 - 0.01) \times 100 = 0.956$ ml. of 0.1 *N* acid for each additional 0.01 microgram of calcium pantothenate. This figure is much higher than corresponding values which may be calculated in the same manner from the data given in previous papers—viz., 0.56 (*13*), 0.49 (*8*), and 0.0715 (*7*) ml. per 0.01 microgram. Thus, for example, in the present method the bacteria produce about 14 times as much lactic acid for a given increment of pantothenic acid as they do on Medium C of Light and Clarke (*7*). These calculations apply only to the recommended assay range in each case—i. e., to conditions under which pantothenic acid supposedly is the only factor limiting acid production.

Table VII. Recovery of Added Pantothenic Acid

Sample	Treatment	Recovery
		%
Dried yeast 110	Clarase	102
Dried human feces	Direct	99
Oil-packed sardines	Direct	103
	Clarase	90
Canned beets	Direct	98
	Clarase	100
Canned peas	Direct	92
	Clarase	101
Canned pineapple	Clarase	100
Clarase	...	99

It is obvious that a large discrepancy still exists between the pantothenic acid content of the yeast samples in Table II as determined by the present microbiological method and by the chick assay. What the cause of this discrepancy may be, and whether it will persist when a large variety of other natural materials are assayed by improved animal methods, must be left an open question at present.

Summary

The procedure for carrying out the microbiological determination of pantothenic acid has been modified by additions to the basal medium and improvements in the method of growing inoculum. The modified method is capable of measuring smaller amounts of pantothenic acid and gives perfectly concordant results at increasing levels of sample.

The apparent increase in pantothenic acid following enzyme digestion as measured by the original method is shown to be largely due to the liberation of fat-soluble and water-soluble stimulants. Methods of avoiding these effects have been worked out, and an enzyme treatment which results in an actual increase of free pantothenic acid in many samples is described.

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Glass Standards for Fluorometric Determinations

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THE application of fluorometric analysis is increasing greatly in the fields of microscopy and biochemistry. The methods offer special advantage, since they make it possible to determine quantities as small as fractions of micrograms. To carry out these analyses, a new class of instruments of the fluorophotometer type has become popular. In operation, these depend on the evaluation of the brightness of the light emitted as a result of fluorescence, the irradiating source usually being confined to the near ultraviolet or visible blue region of the spectrum.

In order to secure the best performance with these instruments, it is necessary to calibrate them with some type of standard. Heretofore, it has been customary to use standard solutions having the proper color and intensity of fluorescence. These are tedious to prepare, as the substances must be weighed accurately and the solutions must be diluted by means of very precise pipets, burets, and measuring flasks.

Furthermore, these standard solutions are difficult to maintain in standard form, as many of them fade or decompose under the influence of light. In one case, that of thiamine hydrochloride [vitamin B_1 (*3*)], the standard has been a solution of either quinine sulfate or thiochrome of proper concentration. Quinine sulfate is now difficult to obtain, and furthermore, both solutions tend to fade. Therefore, standards of fluorescent glass have been used for fluorescent analysis—for instance, a block of uranium glass is furnished with the Pulfrich photometer as a fluorescent standard.

Strength of Fluorescence

Most transparent glasses exhibit strong fluorescence which is characteristic of the particular sample. This does not mean that all melts of a given type of glass will fluoresce in the same manner. The strength of fluorescence is primarily dependent on the chemical constitution and the previous history of the particular glass melt of which it is a part. Trace elements, time and temperature of melting, annealing, cooling, etc., all affect the fluorescence which any glass may display under irradiation. In general, however, a type of glass will always show the same color in greater or less degree.

It is essential that the glass standards be and stay constant. Beese and Marden (*2*), of the Westinghouse Lamp Division, Bloomfield, N. J., found that fluorescent canary glass fatigued when exposed to continuous ultraviolet radiations—that is, the loss in fluorescence increased with time and with intensity of irradiation. The glasses used for standards, however, did not show this fatigue. Experiments made over a long period of time have proved that these standards are perfectly constant and will give consistently reproducible values.

In the effort to find glass of suitable coloration and brightness, by which liquid standards could be replaced, a large group of optical and ophthalmic glasses has been studied. From these studies, it has been possible to select individual melts of particular types of glass which are suitable as standards in the fluorometric determination of thiamine hydrochloride (vitamin B_1) and riboflavin [vitamin B_2 (*1*, *4*)]. These are now manufactured in the form of blocks and will take the place of the cuvettes normally used to hold these standard solutions, thereby effecting a substantial saving.

When compared against a 1 microgram per milliliter standard of thiamine hydrochloride the selected glasses give a value of about 0.5 or 1.0 microgram per milliliter. Another type of glass which is available in various intensities of color gives a range of 0.1 to 0.5 microgram per milliliter for riboflavin.

Wave-Length Bands

The wave-length bands of the fluorescent light produced by the various standards have been determined with a wave-length spectrometer (Table I). The experiments were made with the proper filters used for the determination of vitamins B_1 and B_2. For comparison purposes, the wave-length bands of the standard solutions of quinine sulfate, thiochrome, and riboflavin are also given.

No attempt is made in the preparation of these standards to provide calibration factors. Since this is primarily dependent on the individual instruments, it is left to the user to make his own calibration against standard solutions of thiamine hydrochloride or riboflavin in the instrument in which the standards will be used. They have the approximate values given above, but vary somewhat as new melts of the selected glass are substituted for the original. Once such a standard has been properly calibrated by means of accurately known solutions and under prescribed working conditions, it will serve as a permanent secondary working standard against which the particular instrument may be quickly and easily checked at any time.

Table I. Wave-Length Bands of the Fluorescent Light of the Glass Standards and Standard Solutions

Standards	Wave-Length Band Å.
For Vitamin B_1	
For irradiating light beam: Corning filter, Red Ultra No. 5840. For fluorescent light: Corning filter, Noviol Shade A No. 3389 and filter Light Shade Blue Green No. 4308; slit width 0.1 to 0.2 mm.	
Glass standard I (approximately 0.5 microgram of thiamine per ml.)	4150–5530
Glass standard II (approximately 1 microgram of thiamine per ml.)	4130–5740
Standard solution of quinine sulfate (1 microgram of thiamine per ml.)	4130–5600
Standard solution of thiochrome (1 microgram of thiamine per ml.)	3950–5920
For Vitamin B_2	
For irradiating light beam: Corning filter, Violet No. 511 and Noviol Shade A No. 3389. For fluorescent light: Corning filter, Noviol Shade D No. 3384; slit width 0.2 to 0.3 mm.	
Glass standard III (approximately 0.1 microgram of riboflavin per ml.)	4640–6010
Glass standard IV (approximately 0.5 microgram of riboflavin per ml.)	4650–6170
Standard solution of riboflavin (0.1 microgram of riboflavin per ml.)	4650–5980
Standard solution of riboflavin (1.0 microgram of riboflavin per ml.)	4620–6290

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INDUSTRIAL AND ENGINEERING CHEMISTRY

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INFRARED SPECTROSCOPY
Industrial Applications

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Infrared spectroscopy has proved a powerful tool in solving problems of organic chemistry and finds a widening field in industry. The theory of infrared absorption and its relation to molecular structure are discussed to provide the essential background for detailed descriptions of techniques useful in analysis. Finally a library of 363 representative spectrographs of organic compounds taken in the rock salt region of the infrared spectrum is presented for comparison with those obtained from unknown compounds under investigation.

THE last half dozen years have seen a rapid rise in the contributions of physicists to the chemical industry. Many important problems, which had previously been attacked solely by chemical methods, are now being studied and solved by the methods of both physics and chemistry or by the tools of physics alone. Physical instruments in increasing variety—among them optical and electron microscopes, mass spectrometers, Geiger counters, polarographs, colorimeters, and optical spectrometers of many kinds—are proving more useful every day as their potentialities and capabilities are tested and proved.

The approach of the physicist to a problem is inherently different from that of the chemist. The physicist concentrates upon the physical characteristics of the material itself, its absorption spectrum, x-ray diffraction pattern, exact mass, electronic configuration and properties, whereas the chemist studies the material in reaction with other substances. From these reactions the chemist deduces a wealth of information relating to the original material in question. His apparatus in general is relatively simple. On the other hand, the physicist may require elaborate apparatus of special design, but once this apparatus is constructed, its applications are versatile and the physicist's methods become direct, accurate, rapid, and usually require only small samples. Fortunately, the results obtained from these two methods of approach supplement each other and suffice in most cases to furnish the desired information.

Whether or not one agrees that this is "a physicist's war", certainly it is a time when physical instruments are of enormous usefulness. Devices capable of automatic operation have already proved their merit in practically every field of endeavor. In the chemical industry, today more than ever before, is the need for speed and accuracy keenly felt. The urgency of the demands for multiplied production and the stringent shortage of manpower have necessitated abbreviation of the usual sequence from the research laboratory through the pilot plant to the final production. Short cuts must be taken and sometimes the pilot-plant stage must be eliminated entirely. The production engineer must have immediate and continuous checks on the quality of his product in order to duplicate efficiently the results of the research laboratory. Delay in obtaining analytical results quickly might lead to the waste of large quantities of valuable raw materials and products. Here the saving of time by the physicist's methods is proving invaluable. But here, too, the problems of the industrial physicist are greatly multiplied. He must not only adapt his tools or instruments to solve specific problems under ideal conditions in the research laboratory, but he must also modify them to function dependably under conditions of production analysis and control.

A typical application of physics on a widening scale in industry is infrared spectroscopy. One of the first industrial infrared spectrometers was constructed in this laboratory in the latter part of 1936. Numerous applications of this instrument have been eminently successful and have become familiar to a large number of chemists. Daily, this tool is performing analyses impossible by any other method. More common analyses are being completed in a few minutes which previously required hours.

[1] Absent on leave.

Interest in the potentialities of this physical tool has become so widespread that infrared spectrometers may now be found in the laboratories of an impressive list of American companies, and the time will soon arrive when such instruments will be standard equipment in most laboratories and plants.

The underlying basis of applied infrared spectroscopy is the fact that practically all organic substances possess selective absorption at certain frequencies in the infrared portion of the electromagnetic spectrum. Experimentally, as will be shown below, a spectrometer is used to determine the per cent transmission or absorption of the sample at a series of narrow frequency intervals throughout a chosen part of the spectrum. A plot of these transmission or absorption values versus frequency or wave-length units constitutes an infrared spectrum, which is characteristic of the sample being studied and which may be used to describe the sample in much the same manner as boiling point, refractive index value, or melting point. Because the individual absorption bands of which such spectrum is composed arise from the mutual motion of the atoms within the molecule, these spectral characteristics bear a direct relationship to the atomic masses, the molecular configuration in space, and the binding forces present.

Applications of infrared spectroscopy can be divided into two general categories, qualitative and quantitative.

QUALITATIVE APPLICATIONS OF INFRARED SPECTROSCOPY

(a) "Fingerprinting" of compounds

The infrared absorption spectrum of a given compound is a unique fingerprint which cannot be duplicated by any other compound. This characteristic is invaluable in identifying unknowns. Once a large library of absorption curves for known compounds has been amassed, frequently only minutes are required to match the absorption spectrum of an unknown with one of the known curves. Even in cases where no satisfactory match is found, assurance that the unknown is not one of a list of known compounds is useful information.

Since the samples required are small and are in no way damaged or harmed, this method of analysis is particularly useful in connection with chemical research where limited amounts of material are available.

(b) Recognition of specific chemical bonds, linkages, or groups

Comparisons of spectra of large numbers of pure compounds have shown that absorption bands occurring at certain frequencies can be correlated with certain bonds or atomic groups within the molecule. For example, a glance at an absorption spectrum gives an indication of the presence or absence of such groups as O—H, N—H, C≡N, C=O, CH_3, etc. Observed absorption characteristic of hydrocarbon unsaturation can be ascribed to either aliphatic or aromatic structures. More particularly, the position of the absorption band of a carbonyl group in general indicates whether it forms part of an anhydride, ester, ketone, aldehyde, or acid. The appearance of the "methyl band" distinguishes between the terminal methyl groups of a straight chain and an isopropyl or tertiary butyl group. Although all the structural units of a sample may not be determined in this manner, at least a considerable number of the more common ones can be identified.

(c) Gross structural features

The appearance and analysis of the absorption spectrum can often be used to determine or verify the spatial configuration of the atoms within a molecule. Examples are cis-trans or ortho-, meta-, and para-isomerism, the planarity of the aromatic ring, keto-enol tautomerism, hydrogen bonding, etc.

(d) Qualitative analysis of mixtures

Except in special cases of strong intermolecular interaction, the absorption spectrum of a mixture is equivalent to a simple superposition of the spectra of its individual components. Hence it is possible, by comparing the spectrum of a mixture with the library of spectra of pure compounds, to determine not only the components but also their relative concentrations.

QUANTITATIVE APPLICATIONS OF INFRARED SPECTROSCOPY

(a) Analysis

A mixture of materials can be analyzed quickly and accurately so long as the components present in the mixture are known. From a study of the spectra of the known compounds, it is usually possible to find a frequency at which only one component possesses strong absorption. By setting the spectrometer at this particular frequency and comparing the absorption of an unknown with that of known prepared standards, the amount of the particular component in the unknown can be determined. Usually another characteristic frequency can be found for each of the other components. This rapid method, modified suitably to meet existing conditions, has been applied with an accuracy of 1 per cent or better to a great variety of mixtures from simple ones of two hydrocarbon components to complex mixtures containing up to six terpenes. It is particularly useful for analyzing close boiling isomeric mixtures for, as mentioned above, the absorption spectra are functions of geometrical configuration and are not related to boiling points. A characteristic curve of absorption versus concentration shows the method to be particularly accurate for measuring small amounts of impurities, such as water in oils, oxidation products, etc.

(b) Measurements of reaction rates

The principle outlined above can be used for measuring rates of reaction or polymerization as a function of temperature, pressure, or the catalyst used. Samples may be taken from the reaction chamber at suitable time intervals, and the amounts of the reactants consumed and new products formed quickly determined. Since the amount of sample required is so small (less than 1 gram) its removal from the reacting mass has a negligible effect on the reaction.

(c) Determination of thermodynamic data

A knowledge of the exact values of the infrared absorption frequencies provides part of the information needed for a mathematical calculation of the thermodynamic constants of the material.

(d) Process analysis and control

The analytical methods described can be used in the plant as well as in the research laboratory. Small, sturdy spectrometers which can be set at fixed frequencies have been constructed for such work. Frequently, such control analyses may be made from a by-pass in the production line to give a continuous record of the concentration of one component as a function of time. The output of these spectrometers is an electrical current, which may be used to provide automatic control through suitable relays.

The basic methods described in this paper have been in use in these laboratories since 1937, and are considered to be especially valuable in the present emergency. This paper provides working descriptions of the various experimental techniques involved and a brief discussion of the theoretical basis of infrared spectroscopy. Within the scope of a single paper, however, no attempt can be made to present a complete survey of this field, nor to describe each of the many types of instruments now in use. Rather, the content is restricted to a discussion of the applica-

ions made in this laboratory. Obviously, one of the fundamental requirements for the successful application of infrared spectroscopy is a knowledge of the absorption spectra of a large number of pure compounds. Accordingly a library of 363 spectra has been included.

EXPERIMENTAL EQUIPMENT AND TECHNIQUE

A graph of the complete electromagnetic spectrum is given in Figure 1. Throughout the spectrum the fundamental relationship holds: $\lambda\nu = c$, where λ is the wave length of the radiation, ν is the frequency of vibration, and c is a universal constant, the velocity of light. Because of the various interactions of electromagnetic radiation with matter, each portion of the spectrum is of interest and value to the industrial physicist.

The infrared portion of the spectrum extends from the long wave-length limit of sensitivity of the human eye to the region of ultra short radio waves—that is, from about 7500 Å. (0.75μ) to about 350,000 Å. (350μ). The units most often used in designating portions of the infrared spectrum are: wave-length unit, micron or μ (1μ = 10,000 Å. = 10^{-4} cm.); and so-called frequency unit, wave number or cm.$^{-1}$ [1 cm.$^{-1}$ = 1/λ(cm.)]. The various subdivisions of the infrared spectrum are shown in Figure 1 with dotted lines and are described above the graph. Within very approximate limits these are: the photographic region from 0.75μ to 1.3μ; the overtone region from 1.3μ to 2.5μ; the fundamental vibration region from 2.5μ to about 25μ; and the rotational region from 25μ to 350μ. Although each is important and capable of yielding much valuable information, primary interest centers in the fundamental vibration region. Because of the limitations of radiation sources and prism materials, a portion of this range, from 2.5μ (4000 cm.$^{-1}$) to 15μ (667 cm.$^{-1}$), has been most thoroughly investigated.

The infrared spectrometer used in work in this field consists essentially of: (1) a source emitting a continuous range of wave lengths desired; (2) a dispersing means[2] to spread out this radiation in order to provide narrow wave-length bands at accurately known wave-length positions; (3) a means for interposing a sample of suitable thickness into the path for this radiation; and (4) a detector and amplifier to measure accurately the intensity of radiation in each narrow band. Indicative of the difficulties of infrared technique is the fact that no standardized research spectrometers are available today. In other branches of spectroscopy, it is customary to mention a medium Hilger, a large Bausch & Lomb quartz spectrograph, or a General Electric recording spectrophotometer and let a reference to the literature suffice as a description of the instrument. In contrast to this, the various infrared laboratories use instruments constructed according to the individual ideas, designs, and demands of each investigator. Unfortunately, this paper cannot include descriptions of all the various types of instruments in use, nor can it single out any one as being the best. However, a discussion is given of the spectrometer which was used in obtaining the absorption spectra given below.

THE SPECTROMETER

A schematic drawing of the optical path of the spectrometer is shown in Figure 2. The source is an electrically heated commercial Globar (a silicon carbide rod) having a power consumption of about 400 watts at 115 volts controlled by a voltage regulator. The Globar is thinned in the central section in order to raise the intensity of radiation from the actual source area. The radiation from this source is reflected by the plane mirror, M_1, to the spherical mirror, M_2, which focuses the beam at the position marked "cell". The sample is introduced here by means of an electrically driven way with three stop positions so that a shutter, absorption cell, or a control cell can be placed in the same position in the light path. The radiation transmitted through the cell is then focused by M_3 on the entrance slit at S. S is a double slit having a common center and two movable outer jaws. The entrance slit is curved to compensate for the image curvature introduced by the prisms. The radiation from S enters the spectrometer proper through a slot in the plane mirror, M_5, is rendered parallel by the spherical mirror, M_4, whose focal length is 40 cm. The beam of radiation is given its first dispersion on passing through the two prisms, and is then reflected back through the prisms by the plane Littrow mirror, M_6, for a second dispersion. The dispersed beam traverses the path from P_1 to M_5 to M_4, through the slot in M_5, and passes out through the exit slit at S. The mechanical widths of the two slits control the width of the spectral band and the wave length or frequency of the band center is determined by the rotational setting of the Littrow mirror, M_6. The narrow band of energy emerging from S is then reflected at a small angle by the plane mirror, M_7, to the elliptical mirror, M_8, which focuses a greatly reduced image of the exit slit, S, onto a compensated, vacuum thermocouple. The entire instrument, except for the source and the absorption cell, is housed in an airtight aluminum case whose interior is freed of water vapor and carbon dioxide. For this purpose, air purified over large quantities of phosphorus pentoxide and soda lime is supplied at intervals by a circulating system. The radiation from the source enters and leaves the spectrometer case through rock salt windows.

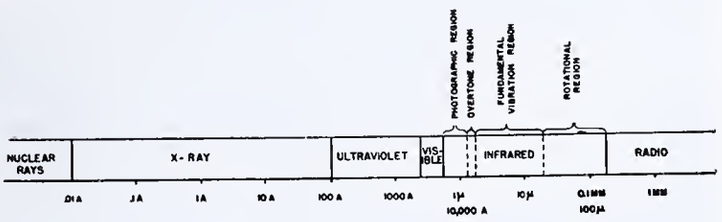


FIGURE 1. GRAPH OF THE ELECTROMAGNETIC SPECTRUM

Since these infrared radiations cannot be photographed and there is no photoelectric element sensitive to this part of the spectrum, the detector must of necessity be some type of thermosensitive element, such as a thermocouple, bolometer, or radiometer.

The order of magnitude of the energies involved illustrates the difficulties encountered in infrared spectroscopy, and accounts for the slow progress in this field as compared with other branches of spectroscopy. The average energy in the dispersed beam produces a voltage at the thermocouple of about 1 microvolt (10^{-6} volt).[3] Since the thermoelectric coefficient of the elements used is of the order of 100 microvolts per degree centigrade, this represents a temperature difference between hot and cold junctions of the thermocouple of roughly 0.01° C. Since, as will be seen later, it is necessary for practical purposes that energies of this order of magnitude be measured with an accuracy of 0.5 per cent, the limit of error must be not greater than 5 × 10^{-5}° C. Small changes in ambient temperature are minimized by using a two junction couple connected in series opposition, only

[2] The dispersing medium may be either a grating or a prism. A grating provides greater dispersion but its use involves more experimental difficulties because of the necessity of eliminating higher order radiation. For this reason prisms are more generally used in industrial spectroscopy. A list of prism materials together with their long wave-length transmission cutoffs is given (glass 1.5μ, quartz 3μ, lithium fluoride 5μ, fluorite 9μ, sodium chloride 15μ, and potassium bromide 25μ). Ideally, best dispersion is obtained by using one material up to its cutoff and then switching to the next material. For a single prism instrument, rock salt is most generally used because it provides the best combination of dispersion and accessible region.

[3] Electronic amplification of this output is difficult because of the low resistance (10–20Ω) and the slow period (one second) of the average thermocouple.

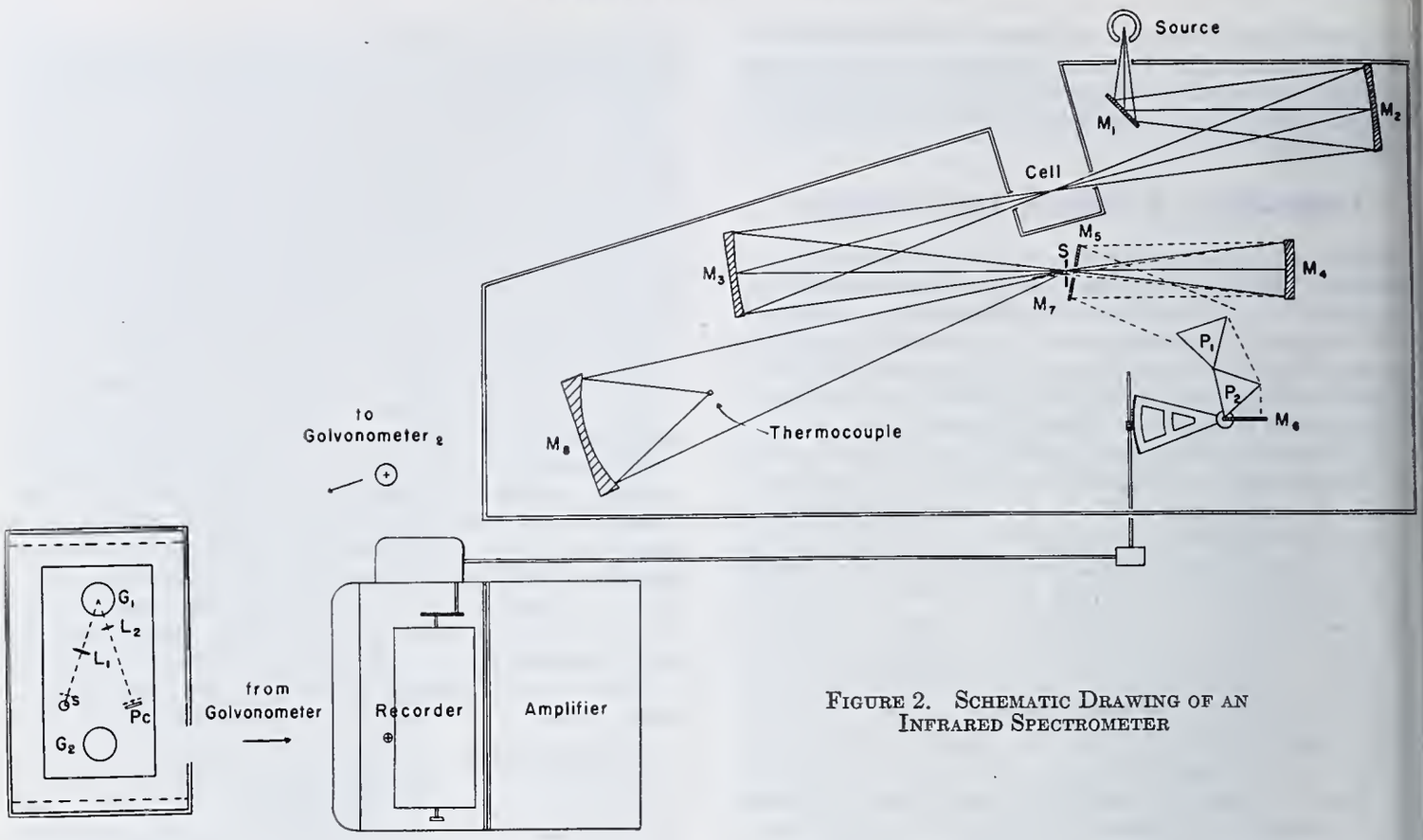


FIGURE 2. SCHEMATIC DRAWING OF AN INFRARED SPECTROMETER

one of the hot junctions being illuminated by the radiation being measured. These values illustrate the excessive care necessary to provide adequate thermal and electrical shielding and to prevent the couple from "seeing" any stray radiation.

To measure accurately the small output of the thermocouple, some suitable form of amplification is essential. A simple method of obtaining this magnification is the conventional photo relay amplifier shown in Figure 2. The voltage from the thermocouple is applied to the primary galvanometer, G_1 (L & N, 0.17μ volt per mm., 3.5 seconds). A focused beam from a headlight bulb, s (operated from a large capacity storage battery), is reflected from the primary galvanometer mirror onto the edge of a barrier layer cell, P_c. A small deflection of G_1 increases the illumination on P_c and proportionately increases the current from P_c, which is fed directly into the recording galvanometer G_2 (L & N, 0.003μ volt per mm., 2.5 seconds). By this method any desired degree of angular amplification up to the Brownian motion limit of G_1 can be achieved. In practice, depending upon the stability of the galvanometers, an amplification factor between 0 and 200 is generally used.

The final spectrogram is obtained by recording the deflections of G_2 with an automatic pen recorder[4] built in this laboratory. A pen is mounted on a carriage which holds a twin cathode photoelectric cell (such as an R. C. A. 920). The light beam reflected from G_2 is interrupted at sixty cycles and is focused symmetrically on the two cathodes so that the outputs of the twin photo cells are equal. Any slight deflection of the beam from G_2 upsets the balance between these outputs. This difference is amplified by one of two Thyratron circuits and fed into a split-field, series-wound motor so geared as to drive the carriage in a direction to restore the balance. Thus the pen carriage follows accurately the deflections of the reflected beam from G_2 and draws, automatically, a continuous record of the galvanometer deflections. This record is drawn on a 12 × 20 inch chart, carried on a rotating drum driven by the same synchronous motor which controls the frequency settings of the Littrow mirror of the spectrometer proper. Suitable calibration marks are made on the record by a small solenoid which deflects the pen at specified frequencies. This solenoid is actuated by a microswitch which is closed once during each rotation of the Littrow shaft. A Veeder counter operated by the same shaft indicates the frequency setting at all times.

In practice, the spectrometer is set at a desired frequency and the slit width and amplification factors are fixed to give the desired spectral slit width and a full scale galvanometer deflection, respectively. The sample is then interposed in the path of the beam, the position of the pen for zero energy is marked on the chart by momentarily inserting an opaque shutter in the light path, and a switch is finally thrown to start the motor. The actual record is a graph of galvanometer deflections as ordinates versus frequency units as abscissas.

This record can be transformed into a per cent transmission graph by a series of calculations using an energy curve covering the same spectral region. In many studies this conversion of the original records to transmission curves is unnecessary.

PREPARATION OF SAMPLES

Samples can be studied as vapors, liquids, solutions, or solids. Windows of the absorption cell can be made of any material which is reasonably transparent in the region of the spectrum employed. In addition to the materials already mentioned as being suitable for prisms, sheets of mica, thin nitrocellulose, or silver chloride can be used in special cases. Examples of typical infrared salt window absorption cells are shown in Figure 3.

Vapors are studied in glass or metal tubes whose ends are closed by cell windows attached with a cement such as Glyptal or Apiezon. Either the pressure in the cell or the length of the cell itself may be adjusted to give the desired degree of absorption.

[4] The basic suggestion for this recorder was obtained from a paper by D. J. Pompeo and C. J. Penther, [*Rev. Sci. Instruments*, 13, 218 (1942)].

In examining liquids, a drop or two of the sample is placed on a plate of salt or other window material on which is laid a U-shaped spacer of thin metal foil to give the required thickness, and then another salt plate is clamped on top of this assembly under uniform pressure. Volatile samples are put in special cells which can be sealed. The average thickness of samples of liquids is about 0.05 mm.

Samples are occasionally studied in solution when the material is a solid or when intermolecular association is to be avoided. Use of this method is greatly restricted since no available solvent is free from absorption throughout the infrared spectrum. Solvents, such as carbon tetrachloride or carbon disulfide whose molecules are nonpolar and consist of a small number of heavy atoms, are most suitable.

It is possible to get good infrared curves from solids if a thin (ca. 0.03 mm.) plate, continuous film, or very finely divided crystalline sample can be obtained. Various methods are employed for producing these. Resinous samples are prepared by laying down a film of suitable thickness from solution and allowing the solvent to evaporate. Satisfactory samples of rubbery materials may be prepared by softening the material to a gluey consistency with a volatile solvent, spreading a thin film on a salt plate and evaporating the solvent in a vacuum desiccator. If melting does not alter the material, the sample can be fused between the plates of a capillary salt cell and allowed to cool, or the sample can be melted on a hot salt plate and smoothed out with a knife or razor blade into a thin layer. Suitable samples may also be obtained by crystallizing the material from solution. If the average particle size of the crystals formed is approximately the same as the wave length of the radiation used for study, the radiation will be scattered rather than transmitted. To avoid this, the crystals must be either considerably larger or smaller than the wave length being used. Samples of insoluble, infusible materials may be prepared for examination by grinding the substance to as fine a powder as possible, then milling it thoroughly in a straight-chain aliphatic hydrocarbon, such as Nujol. The resulting paste is pressed between two salt plates. The Nujol will absorb strongly in the characteristic region of the CH frequencies but will leave the rest of the spectrum clear for absorption by the sample.

THE ORIGIN OF INFRARED SPECTRA

The atoms of any molecule not at absolute zero are constantly oscillating about their positions of equilibrium. The amplitudes of these oscillations are extremely minute (10^{-9} to 10^{-10} cm.) and their frequencies are high (10^{13} to 10^{14} cycles per second). Since these frequencies are of the same order of magnitude as those of infrared radiations, some direct relationship might be expected to exist between the motions of the atoms within molecules and their effects on infrared radiation incident upon them. Actually those molecular vibrations which are accompanied by a change of dipole moment, so-called "infrared active" vibrations, absorb, by resonance, all or part of the incident radiation, provided the frequencies of the latter coincide exactly with those of the intramolecular vibrations. Thus, if a sample of molecules of a single kind is irradiated in succession by a series of monochromatic bands of infrared, and the percentage of radiation transmitted is plotted as a function of either wave length or frequency, the resulting graph may be interpreted in terms of intramolecular motion. Although these atomic motions at first thought seem to be very complicated, they may be shown by detailed analysis to be

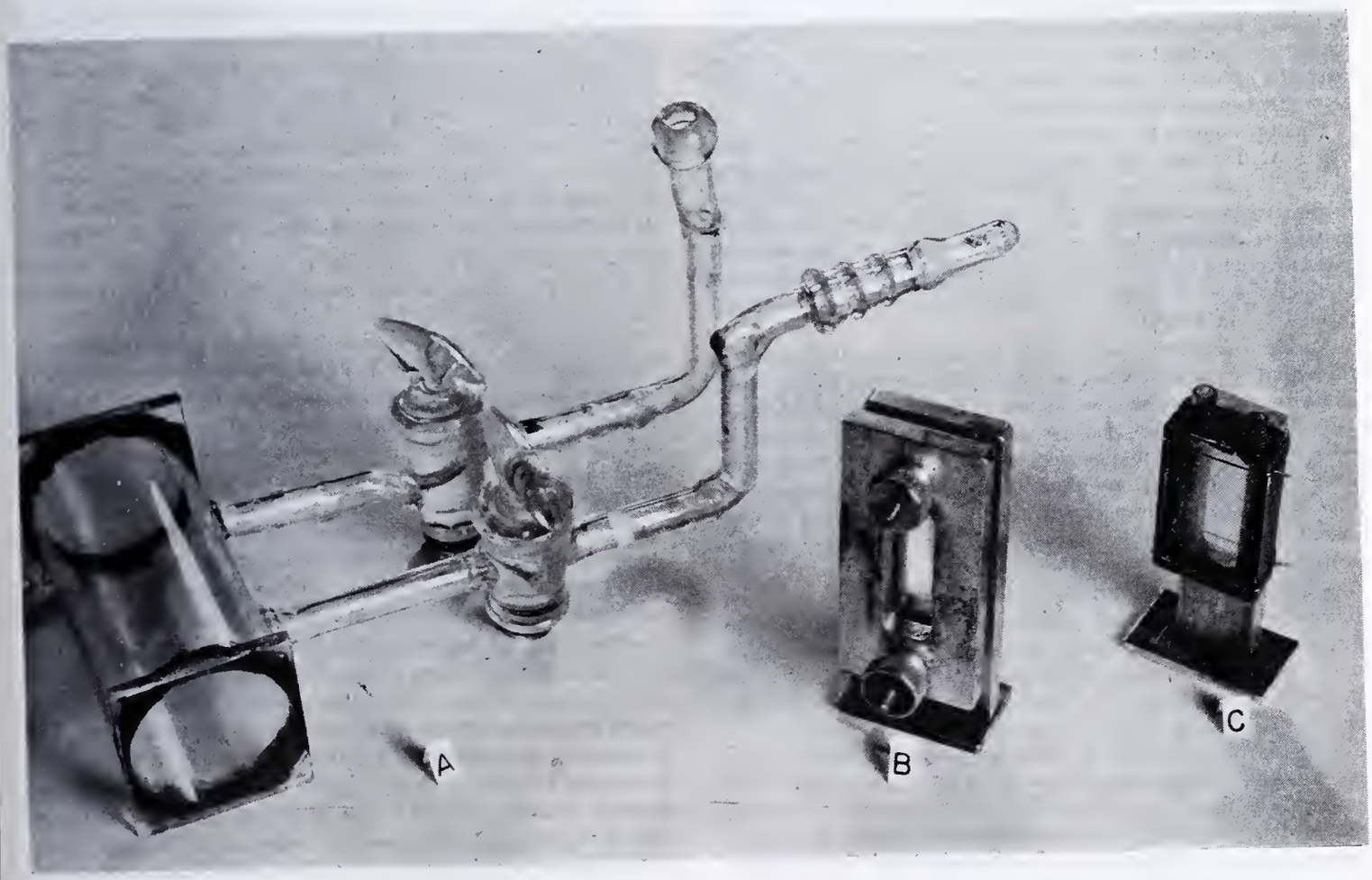


FIGURE 3. TYPICAL INFRARED ABSORPTION CELLS

A. Vapor cell
B. Clamped cell for qualitative studies
C. Fixed cell for quantitative studies

summations of a number of simple oscillations. Each of these simple vibrations is referred to as a "fundamental" or "normal" mode of vibration. Any nonlinear molecule of n atoms can be shown mathematically to possess $3n - 6$ such normal modes, whereas a linear molecule possesses $3n - 5$. A normal mode of vibration is defined as a mode in which the center of gravity of the molecule does not move, and in which all of the atoms move with the same frequency and in phase. Except for cases of accidental degeneracy, each normal mode is independent of the others in that any one can occur without affecting the others. Hence it is possible for all of these vibrations to occur simultaneously and yet for each one to retain its characteristic frequency. An analysis of an infrared spectrum therefore constitutes an analysis of the mechanics of the molecule in question.

The concept of a normal vibration may be clarified by analogy with a mechanical model. Suppose that a model of the benzene molecule, C_6H_6, is constructed by using weights in the ratio of 12 to 1, respectively, for the carbon and hydrogen atoms, these weights being held in proper orientation by suitable springs. Suppose further that the carbon-hydrogen springs are now stretched slightly by moving each of the six pair of weights so that the hydrogens are moved twelve times as far from the equilibrium position as are the carbons. If, now, the weights are released simultaneously, a vibration will occur in which the weights move back and forth along the connecting bonds. The center of gravity of the whole model remains at rest and the weights move only along the line of the connecting springs, since no original impetus was given to the weights in any other direction. This is a characteristic vibration of the model, inasmuch as it has a definite frequency which can be measured by a stroboscope, and it does not excite any other vibration in the model. This stretching motion is exactly analogous to one of the $3n - 6$, or 30, normal modes of the benzene molecule. Another benzene vibration can be visualized readily by pulling the six carbon weights slightly above the plane of the model while the hydrogen weights are pulled twelve times as far below the plane and then releasing all twelve weights simultaneously. Many other characteristic benzene vibrations can be similarly demonstrated.

Finally, suppose that the model is struck a blow with a hammer. At first sight the weights seem to be performing a complicated motion having no apparent relation to the individual modes referred to above. However, if this apparently random motion is photographed with a stroboscopic camera adjusted successively for each of the frequencies of the normal modes, each of these modes of vibration will be found to be faithfully performed by the weights.

An infrared spectrometer plays the same role with respect to the actual benzene molecule as the stroboscope does to the model. Thus, by measuring the frequencies of the infrared radiation absorbed by a substance, the spectrometer determines the characteristic mechanical frequencies of its molecules. Since these molecular frequencies are functions of the atoms themselves—that is, the spatial arrangement, the valence forces, and to some extent the intermolecular forces—the value of infrared spectroscopic information is obvious.

A question naturally arising at this point concerns the possibilities of a mathematical calculation of the normal modes of vibration. Such a calculation, if successfully completed, should make possible a unique determination of the structure of the molecule in question. The correct structure of the molecule would obviously be that whose calculated frequencies correspond exactly to those observed in the experimental spectrum. Theoretically the expected frequencies can be calculated, provided the strengths of all of the interatomic forces are known. Unfortunately, the complexity of such calculations depends on the number of atoms in the molecules and the symmetry of their geometrical arrangement.

Water, for example, with three atoms involves only a third degree equation, and has accordingly been subjected to thorough mathematical treatment. Benzene, although composed of twelve atoms, is so symmetrical that a fourth degree equation is the most difficult encountered. The frequencies calculated for this molecule, too, have been found to agree with experimental values. However, if this geometrical symmetry is completely destroyed by substitution, as in ortho-chlorophenol, a rigorous treatment would require the solution of a thirty-third degree equation. Such a task is out of the question. Accordingly, since most of the molecules of interest to the industrial chemist are quite complex, some other method is required to correlate the characteristics of an observed spectrum with the structure of the molecule. Considerable success in this direction has been achieved by a purely empirical approach.

In order to understand the basis for such an empirical method, it will be well to resort again to a discussion of mechanical molecular models. Consider a model of a molecule containing only one C—H bond, such as chloroform, Cl_3CH. If the C—H spring is stretched and released, the carbon and hydrogen weights vibrate rapidly with a characteristic frequency. The chlorine weights, on the other hand, are so heavy they are almost totally unable to follow the vibration. It is true, at least to a first approximation, that the observed stretching frequency is a characteristic of the C—H spring (bond) and the masses of these two atoms, and is practically independent of the rest of the molecule. Similarly, a bending or deformation can be studied by displacing the hydrogen weight in a direction normal to the axis of molecular symmetry and then releasing it. Again the carbon and hydrogen weights will move characteristically with the remaining weights practically at rest.

These observations lead to the basic premise that, to the extent that atomic forces between a carbon and a hydrogen atom are a function of these two atoms alone, the presence of C—H linkages in a molecule will cause at least two infrared absorptions which are practically independent of the atomic constitution of the rest of the molecule. The validity of this premise has been thoroughly established experimentally. A study of hundreds of molecules containing C—H linkages has shown an absorption around 2900 cm.$^{-1}$ (C—H stretching) and another around 1450 cm.$^{-1}$ (C—H bending). Further verification may be found by a study of the absorption spectra of molecules in which the hydrogen atom has been replaced by a deuterium atom of mass two. A mathematical treatment shows that the C—D frequency should be given by the equation $\nu_{C-D} \cdot \sqrt{2} = \nu_{C-H}$. In such molecules a frequency in the region of 2100 cm.$^{-1}$ (C—D stretching) is observed.

Actually, by using a spectrometer of high resolving power, it can be shown that these C—H frequencies are influenced slightly by the relation of the C—H linkage to the molecule as a whole. For example, the exact frequency value of these absorption bands may be used to indicate the degree of saturation of the carbon atom to which the H is attached or whether the C—H occurs in a CH, CH_2, or CH_3 group.

Although the mathematical approach has been of great value when applied to simple or highly symmetrical molecules, most of the information derived from infrared spectra is obtained by the application of the empirical method. This method consists of comparing the spectra of the largest obtainable number of different molecules having a common atomic group. By a process of elimination it is often possible to find an absorption band whose frequency remains constant throughout the series. The presence, in an unknown, of an absorption at this frequency may reasonably form the basis for a guess that the particular atomic group is present. Confidence in this method can be obtained only by successful applications in a large number of cases.

It must not be assumed from this discussion that it is, or will be, possible to ascribe every observed absorption to a specific atomic group. Indeed, if this were true it would make more difficult the possibility of differentiating clearly between isomeric compounds.

Actually only a few of the observed bands can usually be correlated in this manner. The majority of observed bands arise from normal modes of vibration which are characteristic of the molecule as a whole. These general absorption bands are very sensitive to structural changes, and so furnish us with a "fingerprint" of the molecule. They also make possible the analysis of isomeric mixtures and provide the basis for quantitative analyses of other closely related compounds.

The normal vibrations of a molecule do not account for all of the absorption bands observed in its infrared spectrum. For example, in the far infrared there are absorptions caused by the slower rotations of the molecules or the massive lattice vibrations of crystals. Moreover, throughout the whole infrared region absorptions frequently occur at integral multiples (overtone bands) of the fundamentals, or at frequencies which are equal to the sum or difference (combination bands) of fundamentals. These bands in general absorb very much less strongly than do the fundamentals and consequently must be studied with thicker samples. Since they are so sensitive to the over-all molecular structure, they can sometimes be used more successfully for accurate fingerprinting of molecules and for the analysis of mixtures than the fundamental absorptions.

THE INTENSITY OF INFRARED ABSORPTION BANDS

Except for special cases where intermolecular action occurs, the amount of light transmitted by a sample is governed by Beer's law $I_\nu/I_{0\nu} = e^{-k_\nu cx}$. $I_{0\nu}$ is the radiation incident on, and I_ν the radiation transmitted by, the sample at the frequency ν; k_ν is the absorption coefficient of the sample material at the frequency ν; c is the concentration of a given material in the sample being studied; and x is the length of the optical path or thickness of the absorbing layer of sample. In general, the infrared data shown are presented as a plot of $I_\nu/I_{0\nu}$ as ordinates versus ν (or λ) as abscissas. The factors which influence k_ν have already been discussed. The value of this constant will be appreciable only when ν is an infrared-active fundamental, overtone, or combination frequency. In general, k_ν will have a larger value for polar than for nonpolar compounds. Thus, for example, water, alcohol, and acetone absorb infrared very strongly, while the hydrocarbons absorb weakly. The value of k_ν is fairly insensitive to pressure and, except in special cases, is practically independent of temperature. However, k_ν is not entirely a unique function of molecular structure for slight variations in its values occur between the solid, liquid, and vapor phases of the same molecule. These changes appear as small shifts in the frequencies or intensities of maximum absorption—i. e., maximum k_ν values—and are caused by the varying extent to which the internal vibrations of one molecule are affected by those by its neighbors.

The question may be raised: Why are not infrared spectra plotted as graphs of k_ν versus frequency instead of $I_\nu/I_{0\nu}$ versus frequency? Graphs using $I_\nu/I_{0\nu}$ are functions of both c and x, whereas those using k_ν should be independent of these factors and accordingly more generally applicable. As will be pointed out in a later section, most infrared spectrometers have varying amounts of scattered light and employ varying spectral slit widths in order to attain the sensitivity and stability required in measuring the spectrum with sufficient accuracy. An accurate value of k_ν can be obtained only with an instrument which either has no scattered light or for which the amount of scattered light is known at each frequency. Since k_ν is also a function of spectral slit width, this too would have to be known at each frequency. Furthermore, accurate measurements of sample thickness are extremely difficult. Other difficulties also intervene and until infrared spectrometers have been improved materially and standardized, the conventional plots of $I_\nu/I_{0\nu}$, rather than k_ν, versus frequency are preferable.

QUALITATIVE ANALYSIS

As shown by our previous analogy to molecular models, the atoms of an organic molecule are constrained to oscillate about their equilibrium positions by the valence forces which exist between the atoms. To a first approximation and for purposes of illustration only, these vibrations may be considered to obey Hooke's law. To the extent that this is true, any equation set up to describe these motions must be dimensionally similar to that which holds for simple harmonic vibrations, namely,

$$\nu = \frac{1}{2\pi c}\sqrt{\frac{k}{\mu}}$$

In this expression, ν is the frequency in cm.$^{-1}$, c is the velocity of light, μ is the reduced mass of the vibrating atoms, and k is the force constant which exists between the atoms. The reduced mass, μ, is calculated from the relationship $\frac{1}{\mu} = \frac{1}{m_1} + \frac{1}{m_2}$, where m_1 and m_2 are the relative masses of the vibrating atoms. This constant, k, is related to the vibrations by the equation

$$V = \frac{1}{2}kx^2$$

where V is the potential energy and x the displacement of the atoms from their equilibrium positions. Upon substituting the proper universal constant, the dimensional formula above may be reduced to

$$\nu = 1307\sqrt{\frac{k}{\mu}}\ \text{cm.}^{-1}$$

where k is used as a pure number and μ is expressed in dimensionless atomic mass units.

A brief examination of the literature shows that the value of k for practically all single bonds lies between 4 and 6 $\times$ 10^5 dynes per cm. The corresponding values for double bonds lie between 8 and 12, while those for triple bonds generally lie between 12 and 18 $\times$ 10^5 dynes per cm. For purposes of illustration these values, along with the proper values for μ, may be substituted to calculate the approximate vibration frequencies for a few typical atom pairs.

For the C—H in methane the value of k has been found to be almost exactly equal to 5 $\times$ 10^5 dynes per cm. Since the carbon is so heavy in comparison to the hydrogen, it remains practically at rest, and the value of μ is close to 1. Thus,

$$\nu_{C-H} = 1307\sqrt{\frac{5}{1}} = 2920\ \text{cm.}^{-1}$$

For the C—O in methyl alcohol the force constant is still very close to 5 $\times$ 10^5 dynes per cm., but the value of μ jumps to about 6.85, causing ν_{C-O} to be much lower than ν_{C-H}.

$$\nu_{C-O} = 1307\sqrt{\frac{5}{6.85}} = 1110\ \text{cm.}^{-1}$$

Analogously, for C=O in acetone

$$\nu_{C=O} = 1307\sqrt{\frac{12}{6.8}} = 1730\ \text{cm.}^{-1}$$

Similar calculations for the C≡N group in HCN lead to a value for

$$\nu_{C\equiv N} = 1307\sqrt{\frac{15}{6.5}} = 2000\ \text{cm.}^{-1}$$

In the infrared spectra of these four compounds there are strong absorption bands at 2915, 1034, 1744, and 2089 cm.$^{-1}$ Moreover, a study of a series of molecules containing one or more of

Table 1. Typical Interatomic Characteristics

Atom Pair	Reduced Mass	k	Interatomic Distance Å.	Absorption Frequency $Cm.^{-1}$	Compound
C—C	6	4.50	1.54	993	C_2H_6
C—O	6.85	5.77	1.43	1034	CH_3OH
C—N	6.46	...	1.47	1045	CH_3NH_2
C=C	6	9.77	1.33	1623	C_2H_4
C=O	6.85	12.06	1.21	1744	H_2CO
C=N	6.46	...	1.26	1653	CH_3CHNOH
C≡C	6	17.2	1.20	1975	C_2H_2
C≡O	6.85	...	1.10	2169	CO
C≡N	6.46	16.6	1.15	2089	HCN
C—H	0.92	5.07	1.07	2915	CH_4
O—H	0.94	...	0.96	3683	CH_3OH
N—H	0.93	...	1.00	3370 3315	CH_3NH_2
S—H	0.97	...	1.3	2597	CH_3SH

these linkages shows the characteristic bands approximately at the calculated frequencies. Thus, it is seen that these approximate values of k and μ determine the absorption frequency for a particular atom pair. The slight variations observed in these frequency positions of maximum absorption for molecules that are closely related chemically (hence slight variations in k and μ) make it possible, in many cases, to use the exact frequency empirically as a means not only of telling that a given atomic group is present but also of determining the relation of this group to the remainder of the molecule.

Conversely, once the value of the absorption frequency of a given band has been determined experimentally, it is often possible to calculate the force constant, k, or the binding energy between the vibrating atoms.

Published data show that in general the larger the value of k the shorter the equilibrium distance between the atoms concerned. Table 1 is presented to show the effect of k and μ upon these interatomic distances and upon the frequencies of absorption.

The above discussion is presented in order to give some indication of the theoretical basis for the fact that certain atomic pairs or groups give rise consistently to absorption bands in a well defined region of the spectrum. However, it is to be emphasized that this treatment of extracting a portion of a molecule and treating this portion mathematically is only an approximation and should not be carried to an extreme. The method is applicable to only a very few types of atomic vibrations and it is definitely impossible to start with published data of interatomic distances or force constants and calculate the entire infrared absorption spectrum of a material by this simple means.

Using an empirical approach the authors have examined repeatedly the spectra of many hundreds of organic compounds, and these searches have rarely failed to reveal interesting correlations between bond frequencies and structural relationships. It would be out of place to cite each of these correlations, but a few are given as illustrations of the type of information which may be obtained through careful study of a large number of infrared spectra. It must be pointed out, however, that the curves used in these studies, some of which are reproduced in a later section, were obtained through the use of the large research type spectrometer described above. Users of lower resolving power instruments may experience difficulty in checking some of the correlations indicated below. In view of the inability of any prism spectrometer to resolve completely the structure of complex bands it is frequently true that the shape of a given region of the spectrum is just as important from a qualitative analysis point of view as the exact values of the absorption maxima or transmission minima. In the interest of economy of space, however, we have chosen, in the remainder of this section, to present the illustrations of constant bond frequencies through the use of line graphs rather than full transmission curves. In these graphs the height of the line may be taken as a rough measure of the intensity of the absorption.

(a) High frequency (3μ) region

In the region from 3700 cm.$^{-1}$ to 2500 cm.$^{-1}$ occur the absorption bands arising from hydrogen stretching vibrations, the oscillating motion of a hydrogen atom in the direction of its valence bond. Unfortunately, the dispersion of a rock salt prism is not very good in this region and a lithium fluoride prism or a grating is necessary to make available all the qualitative information which the region offers. However, the type of information which can be obtained from a sodium chloride prism is shown in Figure 4.

"Free" hydroxyl vibrations, where the hydrogen is not affected by any atom except the oxygen to which it is bound, have a characteristic absorption between 3700 and 3500 cm.$^{-1}$ If the material is concentrated so that "hydrogen bonding" can occur, this absorption becomes broader and stronger and moves to lower frequency. It has been shown that there is a relationship between the energy of the hydrogen bond and the frequency shift from "free" to "associated" state. Because the hydroxyl frequency is exceptionally sensitive to the effects of intermolecular association and internal chelation, it is always necessary to consider the physical state of the sample in interpreting this region. Benzyl alcohol, diluted in carbon tetrachloride, shows the free hydroxyl absorption at 3640 cm.$^{-1}$, while in the pure material this band has shifted to a broad deep absorption centering around 3350 cm.$^{-1}$ Under high dispersion these association bands sometimes show discrete structure which probably arises from the types of molecular aggregates involved.

N—H bonds show a characteristic absorption between 3500 and 3200 cm.$^{-1}$ Aniline has two N—H bonds, one at 3445 cm.$^{-1}$ and

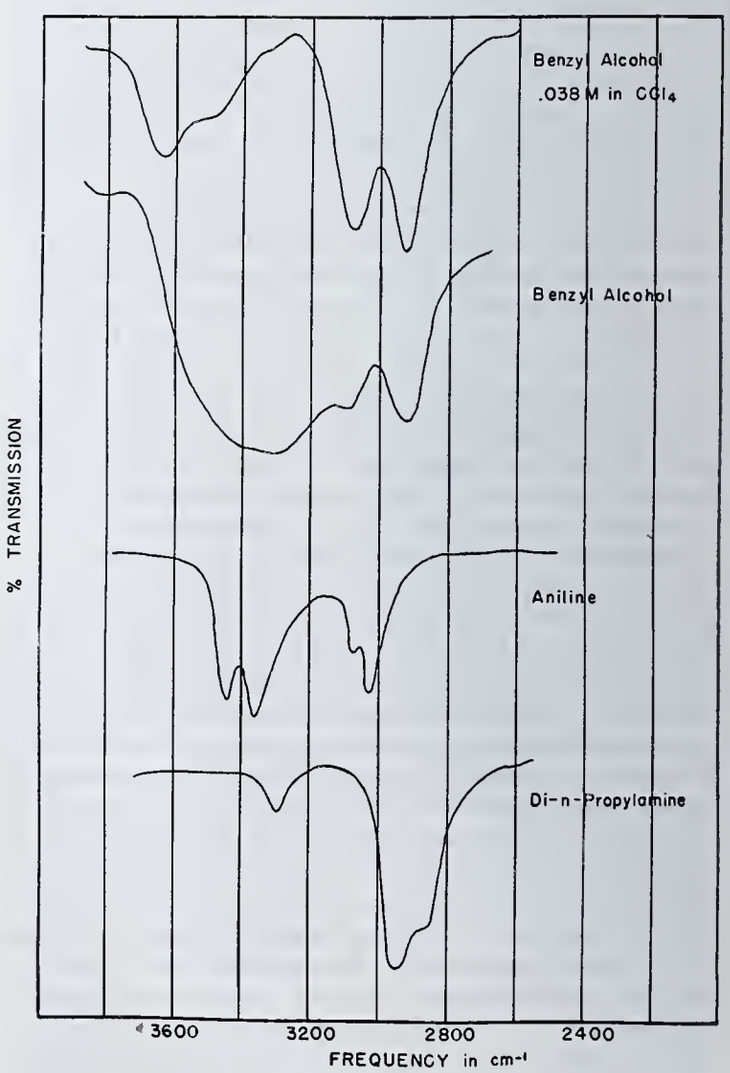
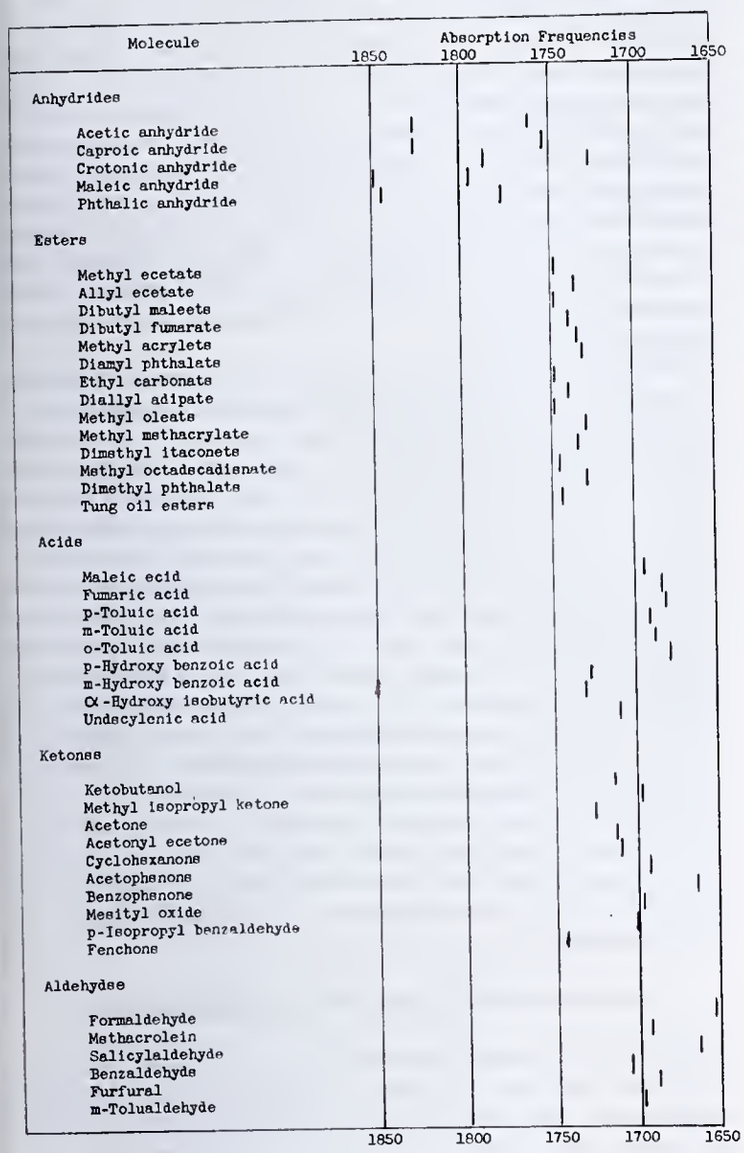


Figure 4. Characteristic Absorptions of X—H Bonds

TABLE 2

C=O FREQUENCIES



the other at 3368 cm.$^{-1}$, because of the interaction between the two hydrogen atoms on the same nitrogen. Di-*n*-propylamine, on the other hand, shows only one absorption band.

C—H frequencies occur between 3200 and 2800 cm.$^{-1}$ This region is subdivided by the characteristic that the band is usually above 3000 cm.$^{-1}$ if the carbon atom is unsaturated or if the hydrogen occurs in highly halogenated compounds. In normal aliphatic compounds, the band usually occurs below 3000 cm.$^{-1}$ In benzyl alcohol both saturated and unsaturated carbons and C—H bands are observed in each region, respectively. In aniline, only unsaturated carbon hydrogen bands occur at 3080 and 3040 cm.$^{-1}$, while di-*n*-propylamine has only saturated C—H at 2950 to 2870 cm.$^{-1}$ With higher dispersion the unsaturated C—H region shows structure characteristic of CH, CH_2, and CH_3 groups.

Another bond of interest to the chemist is the S—H group whose characteristic absorption lies in the region of 2500 cm.$^{-1}$ A study of the simplified Hooke's law treatment of vibrating atoms shows that the reduced mass, μ, of an X—H group is approximately doubled if a deuterium atom is substituted for the hydrogen. There may occur situations where it would be desirable to study separately the behavior of two X—H groups whose frequencies coincide. If it is possible to substitute a deuterium atom for one of the hydrogen atoms, the infrared characteristics of the two bonds will differ to permit individual study while their chemical natures are unchanged.

(b) Unsaturated region

Absorption bands originating in unsaturated bonds between atoms of the second row of the periodic table occur between 2300 and 1300 cm.$^{-1}$

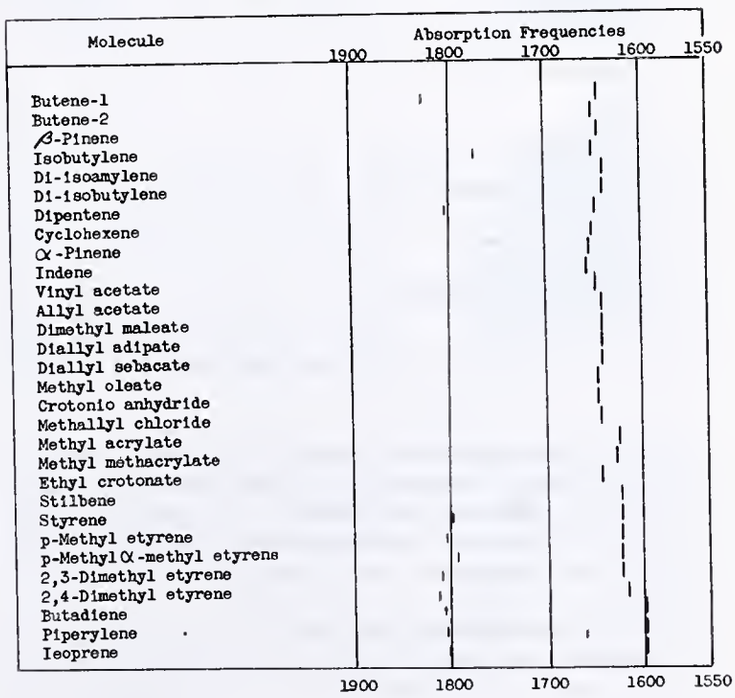
The highest frequencies observed are those of the triple bonds between 2300 and 2000 cm.$^{-1}$ from such linkages as C≡C, C≡N, and the C≡O of carbon monoxide and carbon dioxide. There is little chance of error in ascribing an observed band in this region to a triple bond origin, for the only other fundamental vibrations which can occur here are the above-mentioned X—D vibrations.

Carbonyl frequencies fall between 1850 and 1650 cm.$^{-1}$ as shown by the line graphs of Table 2. To a certain extent these bands fall in a fairly narrow region of the spectrum according to the relationship of the group to the rest of the molecule. The anhydrides usually show a double band with one minimum between 1850 and 1800 cm.$^{-1}$, the other between 1800 and 1750 cm.$^{-1}$ Ester carbonyls are generally in the region from 1750 to 1725 cm.$^{-1}$, aldehydes and ketones from 1725 to 1690 cm.$^{-1}$, and acids from 1700 to 1670 cm.$^{-1}$ These criteria are not always valid, for it can be seen from the graph that low members of a series are often out of line while conjugation tends to lower the characteristic frequencies. Other regions of the spectrum can be used to check the tentative correlation of an observed carbonyl bond. If an acid is suspected, the high frequency region can be examined for hydroxyl absorption. Esters in general show a strong band from 1300 to 1200 cm.$^{-1}$ which probably arises from the single bond carbon oxygen where the carbon is an unsaturated atom. Phthalates in particular show a characteristic triplet at 1275, 1120, and 1070 cm.$^{-1}$

Aliphatic carbon-carbon double bond linkages occur between 1660 and 1600 cm.$^{-1}$ as shown by typical examples in Table 3. It is to be noticed that such bands usually occur between 1660 and 1640 cm.$^{-1}$ when the bond is not conjugated. When the bond is conjugated with an aromatic unsaturation, the frequency drops to 1625 cm.$^{-1}$, whereas, if full aliphatic conjugation occurs, the frequency is nearer 1600 cm.$^{-1}$ It would be well to repeat the point here that atomic motions give rise to infrared absorption only if the vibration causes a change in dipole moment. Therefore, the C=C absorption may be weak or nonexistent if the bond occurs in a perfectly symmetrical molecule such as ethylene, or

TABLE 3

ALIPHATIC C=C FREQUENCIES

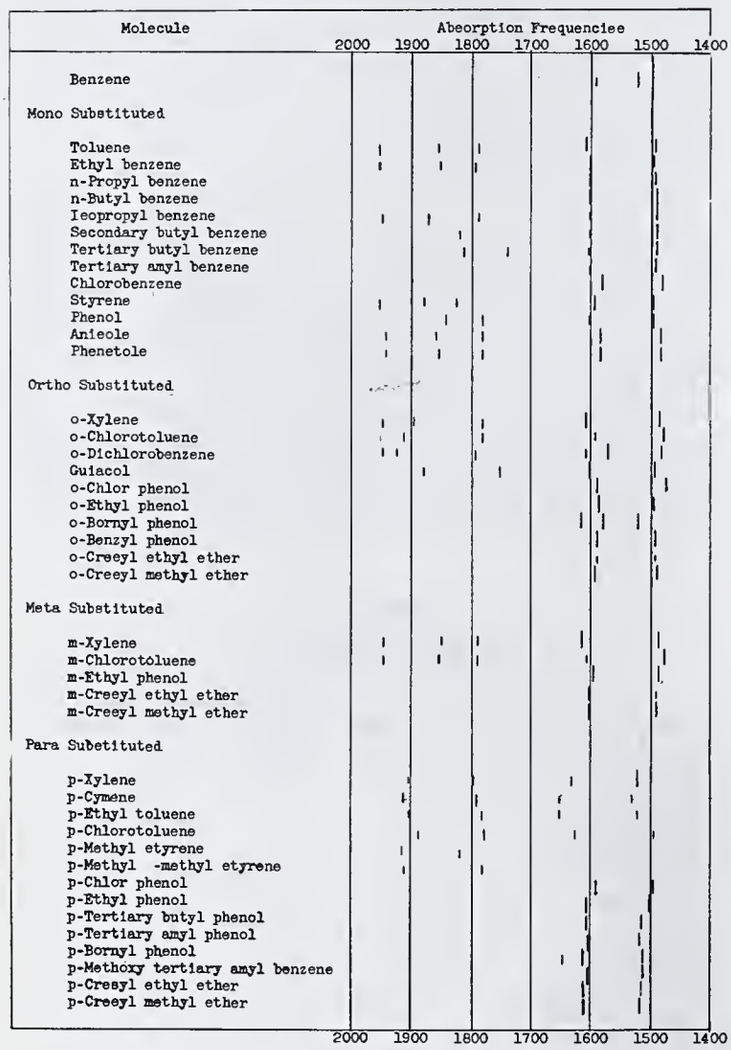


in the middle of a long hydrocarbon chain where the atomic structure at each end of the bond is well balanced.

The carbon-carbon unsaturation of the benzene ring is evidenced by an absorption band near 1600 cm.$^{-1}$ and another near 1500 cm.$^{-1}$ Not only are these bands very characteristic of the phenyl ring itself but their specific positions in conjunction with other bands throughout the spectrum furnish a tentative determination of the manner in which the ring is substituted. There appears to be some correlation between the type of substitution and the position of the overtone bands between 2000 cm.$^{-1}$ and 1700 cm.$^{-1}$ although this evidence is still meager and may be a function of the particular group substituted. In general the 1500 cm.$^{-1}$ band appears to be high for para substituted molecules. Mono substituted aromatic compounds generally have a band from 1075 to 1065 cm.$^{-1}$ and from 720 to 700 cm.$^{-1}$; ortho substituted, 1125 to 1085 cm.$^{-1}$ and 720 to 700 cm.$^{-1}$; meta substituted, 1170 to 1140 cm.$^{-1}$ and 790 to 735 cm.$^{-1}$; and para substituted, 1120 to 1090 cm.$^{-1}$ and 850 to 775 cm.$^{-1}$

TABLE 4

AROMATIC RING FREQUENCIES



Other common unsaturated bands of interest are the C=N group which absorbs near 1650 cm.$^{-1}$ and NO_2 which generally has bands near 1550 cm.$^{-1}$ and 1340 cm.$^{-1}$ In this region bands are also to be expected which arise from water or from unsaturated ring structures whose characteristics are not well known.

Some other characteristic absorptions may be reviewed briefly: The terminal group $H_2C{=}CHX$ has an overtone band near 1800 cm.$^{-1}$ and a CH_2 bending vibration between 1420 and 1400 cm.$^{-1}$ The presence of CH_2 or CH_3 groups in a material gives rise to absorption between 1475 and 1430 cm.$^{-1}$, while a band at 1375 cm.$^{-1}$ is indicative of the grouping C—CH_3. This 1375 cm.$^{-1}$ band is quite characteristic of the type of methyl structure present. For a tertiary butyl group the band is split with a weak component about 1385 cm.$^{-1}$ and a strong one at 1367 cm.$^{-1}$, while for isopropyl groups the band is split into a doublet at 1380 and 1370 cm.$^{-1}$ of approximately equal intensity. C—Cl bonds have a strong band about 750 cm.$^{-1}$ with an overtone at about twice that frequency. A C—O linkage where the carbon is unsaturated has a band between 1250 and 1200 cm.$^{-1}$, as evidenced by esters and aromatic ethers. If the carbon atom is saturated, this band is at a lower frequency, as in aliphatic ethers, near 1100 cm.$^{-1}$

This type of empirical correlation between observed absorption and molecular structure is essential to qualitative analysis and it is here that a great deal of work must be done. At the present time, frequencies above 1350 cm.$^{-1}$ allow fairly definite assignments while those below 1350 cm.$^{-1}$ will necessarily be less definite because these vibrations generally involve all the atoms of a molecule rather than those of a specific part. Moreover, exceptions to any correlation will be found because the exact values of the frequencies are always dependent on the rest of the molecular substitutions or unsaturations, and accidental degeneracies will occur to split an expected single band into a doublet which will confuse the spectrum. However, the information which has already been gained has proved of great value in such applications as indicating the presence or, of equal importance, the absence of specific groups in an unknown material. It will well repay spectroscopists to obtain more data, to group and regroup all available spectra for studying a specific molecular characteristic, and to draw conclusions from these studies which will permit of a standard, rapid infrared approach to the identification of unknown compounds.

QUANTITATIVE ANALYTICAL TECHNIQUES

It has been pointed out previously that the experimental difficulties which have hindered the general progress of infrared spectroscopy have been the lack of strong energy sources and adequate energy detectors. These two weaknesses are most keenly felt in making accurate intensity measurements. It is only natural, therefore, that in the past one of the most practical applications of infrared, its use for quantitative analysis, should have received very little attention compared with its qualitative applications. However, the fact remains that infrared answers the two prime requisites for analysis: that every compound should have a unique characteristic and that it should retain that characteristic on admixture with other materials. The infrared spectrum of an organic material is a unique characteristic of that material, and the infrared spectrum of a mixture of materials is, in almost all cases, an additive function of the spectra of each component in a pure state. These facts, combined with the characteristics of speed, small sampling, and the ability to recover the sample unchanged have impelled the industrial infrared spectroscopist to improve his experimental techniques to the extent that an appreciable percentage of his time is now spent in performing accurate quantitative analyses.

The fundamental rule correlating component concentration with the absorption intensity of that component is the well known Beer's law $I_\nu = I_{0\nu}e^{-k_\nu cx}$ which was discussed under Origin of Infrared Spectra. Theoretically, the application of this law to the analysis of any mixture is quite straightforward. The spectra of each component is obtained in a pure state. By comparison of these spectra, those frequencies are chosen for which one component shows a strong absorption whereas the absorption of the other components is weak. Using pure materials (c = 100 per cent) and known or constant values of x, k_ν is determined for each component at each of these chosen frequencies.

With these calibration data obtained, the analysis of an unknown mixture consists first of determining experimentally the per cent transmission (I/I_0) for each chosen frequency. The logarithm of these transmissions then furnishes a set of linear equations, $\log(I_\nu/I_{0\nu}) = x(k_{\nu a}c_a + k_{\nu b}c_b + \ldots\ldots)$, which can be solved for the desired concentrations c_a, c_b, etc. Theoretically the method is straightforward but actually it may be complicated by experimental difficulties or tedious mathematics in case the number of components is large. Therefore, while it may be considered a rigorous method of analysis, in particular cases it is desirable to seek short cuts and more empirical methods of analysis which will shorten the process without sacrificing accuracy.

It is not feasible to discuss all the possible types of analysis which may arise nor to go into great detail on all the methods of treatment which may be used. Rather, the subject is treated by a consideration of the instrumental difficulties attendant upon infrared analysis and the bearing which these difficulties may have on the method to be used; a brief discussion of the possible methods of handling materials in vapor, liquid, or solid state; and finally, a few illustrations of typical analyses.

There are two main characteristics of the analytical instrument to be used which will influence the method of analysis chosen. The first of these is that an infrared spectrometer is apt to suffer from scattered light. The typical infrared source (an approximation to the black body radiator of Figure 5) throws most of its energy into short wave radiation and it is these short wave components which are scattered most strongly. As can be seen from Figure 5, a black body source at 2000° C. has an intrinsic energy at 1.5μ which is about one hundred times greater than the energy in the 10μ region where a great deal of analytical work is done. The presence of scattered light in a measurement of I/I_0 makes the direct use of Beer's law inaccurate, especially at low values of I/I_0. While it is true that the amount of scattered light in an instrument can be determined as a function of ν, it is difficult to determine the extent to which this factor is reduced when an arbitrary sample is introduced into the light path.

Careful instrumental technique is required to reduce the short wave scattered light to the point where it is an unappreciable percentage of the longer wave-length energy desired. One method is to introduce at the entrance window of the spectrometer a magnesium oxide filter whose particle size is so chosen as to cut off light of frequency higher than the highest frequency used in the analysis. Again, the shutter used for determining the galvanometer reading for true zero energy may be so chosen that it will stop all radiation at the analytical frequency but will transmit a great portion of the energy at higher frequency. By the use of such devices, as well as careful light baffling inside the spectrometer, the factor of scattered light in analysis can be reduced so that it is negligible.

The other analytical difficulty lies in the relation of the spectral slit width used to the width of the characteristic band whose absorption is to be measured. In the ultraviolet and visible regions, the absorption bands studied are generally much broader than the spectral slit width used. Therefore, it is possible for one ultraviolet or visible spectroscopist to publish a value of the absorption coefficient, k_ν, for a pure material, and for another operator to use this value directly with his own instrument.

Unfortunately, this situation does not exist at present for the infrared spectroscopist. Most organic molecules possess absorption bands whose widths at half maximum are of the same order of magnitude as the spectral slit width (10 to 15 cm.$^{-1}$) used to scan the bands. For independent operators to use a published value of k_ν on instruments which are not identical, would require an accuracy of frequency setting and spectral slit width that would exceed normal operating conditions. This means that, until infrared instruments are more standardized and instrumental technique becomes more accurate, published values of k_ν or plots of I/I_0 *vs.* ν can be used as rough values to indicate the possibilities of analysis, but each spectroscopist must determine usable values of k_ν and limits of error as a function of his own instrument.

The accuracy with which an instrument can be made to repeat a frequency setting and spectral slit width within the time which can be devoted to a given analysis, will determine the general method to be used. If these factors can be reproduced, a previously determined working curve of per cent transmission *vs.* component concentration or a value of k_ν can be used safely over considerable time intervals. If the conditions cannot be repeated with sufficient accuracy, it is necessary to measure standard mixtures together with the unknown until the desired degree of bracketing for interpolation is attained.

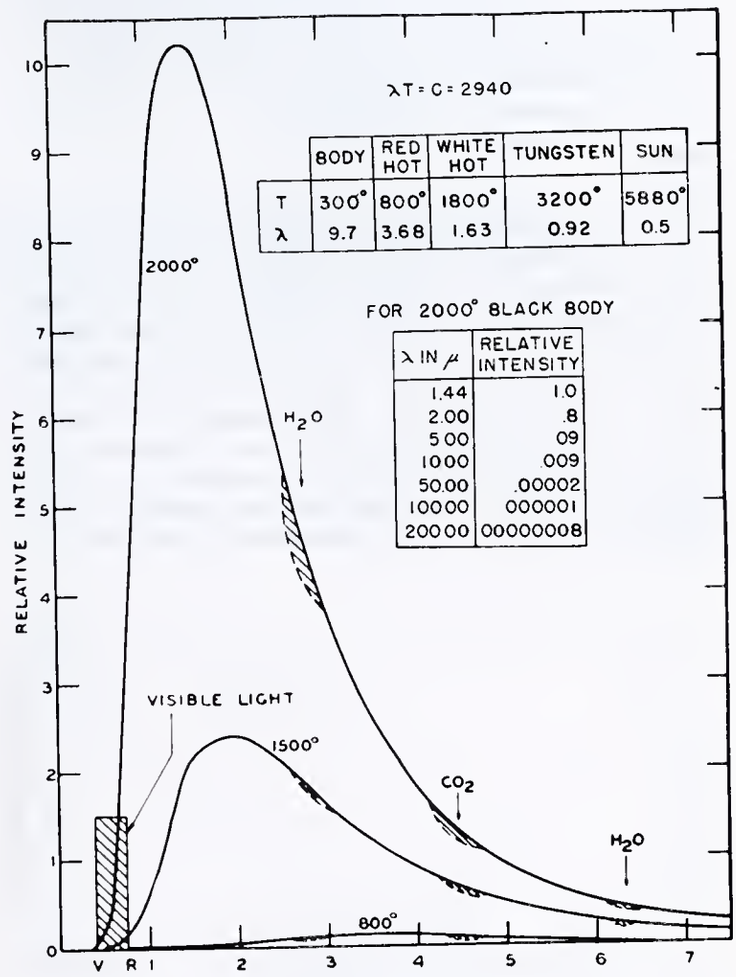


FIGURE 5. EMISSION OF A BLACK BODY RADIATOR

Assuming the possibilities of repeating experimental conditions or running enough standards for correlation, the accuracy of the typical analysis is limited further by the ability of the instrument to measure a per cent transmission. This depends on galvanometer steadiness, sharpness of thermal equilibrium, the stability of the source during the time interval required to measure $I_{0\nu}$ and I_ν, etc. These uncertainties are functions of the instrument only and are fairly independent of the absolute value of $I_\nu/I_{0\nu}$. A study of the graph of Beer's law, Figure 6, shows that a given error in the measurement of $I_\nu/I_{0\nu}$ represents a much smaller error, based on total composition, when the concentration of the component is small than when it is large. That is, spectroscopic analyses are, in general, better at measuring small amounts of impurity than at measuring major components. Therefore, in simple analyses, it is generally better to measure the lesser components directly and obtain the major component by difference, provided all the components present are known.

To a certain extent, a nice analogy can be drawn between the conditions for the physicist's infrared analysis and the chemist's

distillation analysis of a mixture. Each would like to know beforehand all the components involved. In case all the constituents are not certain, the chemist can run a very careful distillation to see if the observed boiling point plateaus correspond to the expected components. The infrared spectroscopist can obtain a careful absorption spectrum of the mixture to check that all observed bands can be correlated with the expected components. The chemist requires the a priori knowledge of boiling points, probabilities of constant boiling mixtures, decomposition, or polymerization in order to predict the possibilities of analysis; the spectroscopist, the a priori knowledge of pure spectra and the possibilities of intermolecular effects which vary with concentration. Where close boiling points are troublesome for the chemist, a lack of strong, well resolved absorption bands creates comparable difficulties for the physicist. Such preliminary information combined with an intimate knowledge of the apparatus available enables each to predict quite well the accuracy with which he can perform a proposed analysis.

Sampling

One important consideration which must be decided before making an analysis is the sample thickness to be used. It may be seen from the dotted lines of Figure 6 that a constant small error in the transmission $[dT = d(I/I_0)]$ results in varying changes in c as T ranges from 0 to 100 per cent. Hence the analysis should be carried out at that value of T for which a given change of dT results in the smallest change of dc/c, or at the value of T for which $1/c\ dc/dT$ is a minimum. Differentiation shows that this results when T is e^{-1} or 37 per cent. Thus it is desirable to adjust the sample thickness so that the transmission at the frequency of a strongly absorbing component should be in the neighborhood of 37 per cent for the average concentration to be expected.

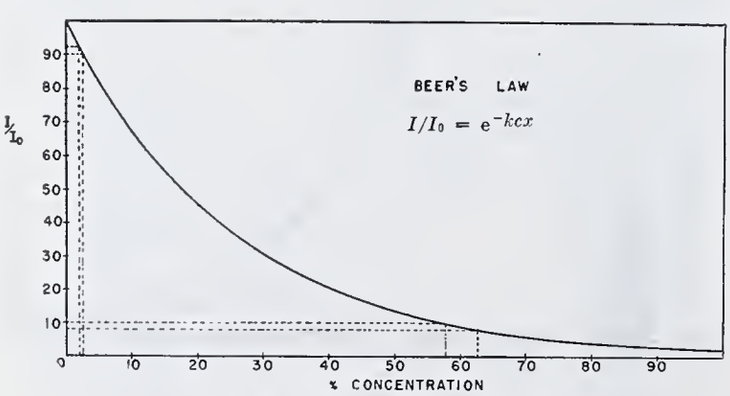


FIGURE 6. GRAPH OF BEER'S LAW

The above considerations apply only to the measurement of a single component in the presence of a nonabsorbing medium. In the case of a multicomponent analysis further study shows that the value of T should be lower than 37 per cent by a function of the absorption coefficients of these other components. Fortunately the function $1/c\ dc/dT$ does not vary rapidly with T, so that no appreciable accuracy is lost if x is so chosen that T lies between 25 and 50 per cent.

In considering analysis of samples in the gas, liquid, or solid state, gas analysis presents the most favorable situation for two reasons. In the first place, the sample thickness, the product of pressure by cell length, can be regulated by a choice of either factor. For any given cell length, the pressure can be varied easily to ensure the best value of T at each frequency. Moreover, since the cell length is of the order of centimeters it can be measured easily and the pressure can be measured accurately by means of a mercury or Octoil manometer. In the second place, vapor analysis is favorable because there is little interference from intermolecular effects. For molecules possessing some degree of geometrical symmetry there are vibrations which do not result in infrared absorption because they do not involve a change of dipole moment. However, in the liquid or solid state, these vibrations may give rise to absorption, inasmuch as the selection rules are modified by intermolecular reactions. It is true that these absorptions are weak but they may become appreciable in using a thick sample to bring out a slight impurity. Therefore, the favorable analytical situation of one component absorbing strongly against a weak background from the other components is more probable in the vapor than in the liquid or solid state. Gas cells generally vary from 1 to 20 cm. and the pressures may run from a few millimeters of mercury to atmospheric pressure (or greater).

The sample thickness for liquids varies from less than 0.01 mm. for very polar materials to 10 mm. to bring out overtone or combination bands. Working with such small dimensions poses the problem of an absorption cell which will repeat its thickness to better than 0.5 per cent. The simplest way to meet this problem is to make a cell of fixed thickness which can be cleaned and refilled. For a given analysis it is not necessary that the sample thickness be known accurately, but it is necessary that it be repeated accurately. Cells for this purpose can be made by clamping two rock salt plates together with an appropriate metal foil spacer to fix the thickness. Holes may be drilled in the top of the cell plates and glass or brass tubes inserted for corking volatile materials. The cells may be sealed by using a mercury amalgamated spacer or by coating with a suitable material such as Apiezon wax, Duco cement, or Glyptal resin. If the liquids are volatile the cell can be cleaned by a stream of dry air; if not, air combined with a suitable volatile solvent can be used. For fixed cells less than 0.05 mm., capillary action restricts the ease of cleaning and refilling. If it is necessary to work with a sample thickness less than 0.05 mm. in order to get the required transmission, it is best to dilute the sample with some material which has weak absorption at the frequencies of interest. However, since all solvents show some infrared absorption, it is usually desirable to use as thin a cell as is compatible with the solubility of the material in question. The cell thickness chosen for a given analysis should give the best compromise of transmission values at the chosen frequencies or more than one fixed cell must be used.

There are occasions where a choice can be made between studying a mixture in a liquid or completely vaporized state. As a rule this choice can be made only after experimenting with each method. While vapor studies have the advantages discussed above, it is also true that values of k_ν vary considerably as the material goes from the liquid to the vapor state and it may happen that an impurity band will show a greater value of k_ν relative to the background in the liquid state.

The sample thickness for solids is of the same order of magnitude as that for liquids. If the solid material can be dissolved in a suitable solvent, the methods of liquid analysis can be used. If it is not soluble, then the problem of repeating a sample thickness becomes so difficult that the time involved would be prohibitive in the general case. There is some possibility of working with known amounts deposited from solution or mulled in Nujol where the same plates and spacer conditions could be repeated. However, this method, at best, is not very satisfactory. The easiest method of attack is to repeat the sample thickness as closely as possible by identical methods of preparation. The analyses can then be made for component ratio by measurements of relative band depths or by including a known amount of some new material to be used as an internal standard.

Typical analyses

A few typical analyses have been chosen to illustrate the various methods of treatment which may be used. Most of these are applications which have been made in this laboratory as actual analyses or to determine the accuracy which might be expected

the analysis were undertaken. The absorption spectra on hich they are based are given in the section on Library of Ref-ence Curves.

DETERMINATIONS OF A SINGLE COMPONENT—i. e., MEASUREMENT AT A SINGLE FREQUENCY

Impurity Analysis

The simplest analysis which is encountered is the measurement f a material for a small amount of an expected impurity. An xample of such an analysis is the determination of cyclohexanone n cyclohexane shown in Figure 7. The obvious frequency for nalysis is the strong carbonyl absorption of cyclohexanone at 718 cm.$^{-1}$ where cyclohexane itself has very weak absorption. Four possible methods of analysis are discussed in the light of ex-erimental conditions and the accuracy desired.

a. Record Comparison. This method of analysis is based pon the automatic absorption records of galvanometer deflec-ion obtained for a series of known standard mixtures. An un-nown can then be determined by visual comparison of the ab-orption record of the unknown with those of the known series. The method is rough at best and can be used only with a record-ng spectrometer, but it is rapid and eliminates the necessity of aving to set the instrument accurately at the absorption maxi-num. A series of such standard absorptions is shown in Figure 7,*A*. The sharp background absorption structure, which is aused by residual water vapor in the instrument, is useful as an nternal frequency calibration standard.

b. Working Curve. A more accurate method of analysis con-ists of measuring the per cent transmission of an unknown sam-ple of cyclohexane, and determining the per cent of cyclohexa-one present by reading directly from a working curve such as hat shown in Figure 7,*B*. This working curve was obtained by etting the instrument at 1718 cm.$^{-1}$, and measuring the per ent transmission of the series of known standards with an auxil-ary galvanometer lamp and scale. With this method the per ent transmission includes the scattered light contribution and he sample cell absorption since the measurement of I_0 is made with nothing in the light path. Hence the accuracy of the nalysis is dependent on the constancy of the cell window ab-orption, which must be checked from time to time.

A study of Figure 7,*B* reveals some characteristics that have een discussed previously. The curve at low concentration is almost a straight line as would be expected from an expansion of e^{-x} where $x << 1$. With a knowledge of the accuracy with which a per cent transmission can be measured, the error in measuring the concentration can be determined from the slope of the curve at any point. Assuming that the error in reading I/I_0 is ± 1 per cent. the corresponding error in concentration is ± 0.01 per cent at 0.1 per cent, ± 0.02 per cent at 0.5 per cent, and ± 0.1 per cent at 2 per cent concentrations, respectively. This shows that the absolute error, dc/c, is a desirable minimum in the neighborhood of 37 per cent transmission where dc/c is equal to 0.02/0.5. The absorption cell chosen (0.2 mm. thickness) should be used when the cyclohexanone is expected to be about 0.5 per cent concen-tration. If the impurity is expected to be considerably less than 0.5 per cent, greater analytical accuracy would be obtained with a thicker cell, whereas for concentrations greater than 0.5 per cent a thinner cell should be used.

c. Beer's Law Graph. In Figure 7,*C* the per cent trans-mission values of 7,*B* have been corrected for scattered light and absorption cell plus pure cyclohexane absorption. A plot of log I/I_0 *vs.* concentration shows a fairly straight line with some deviation at the high concentrations. In this particular analysis there is no gain in accuracy and a loss in time in going to the straight-line log plot. However, there might arise a case in which there is insufficient material to make up a series of standards, or an unknown impurity may be observed which cannot be identi-fied to make possible a series of known standards. In the latter case the amount of impurity in one sample may be taken as an arbitrary unit of concentration. A measurement of this sample and the pure material determines k_ν, the slope of the straight line of Figure 7,*C*. Then the concentrations of this impurity in other unknowns may be measured in terms of the arbitrary unit chosen.

d. Special Methods. It is not strictly true to say that the accuracy of a concentration analysis is always limited by the accuracy of determining a per cent transmission. An exception to this is illustrated by a special method of analysis which can be used for measuring a small concentration of an impurity which does not present a favorable case for analysis—i. e., does not have a strong absorption at a frequency for which the major compo-nent absorbs very weakly. A frequency can be chosen, preferably

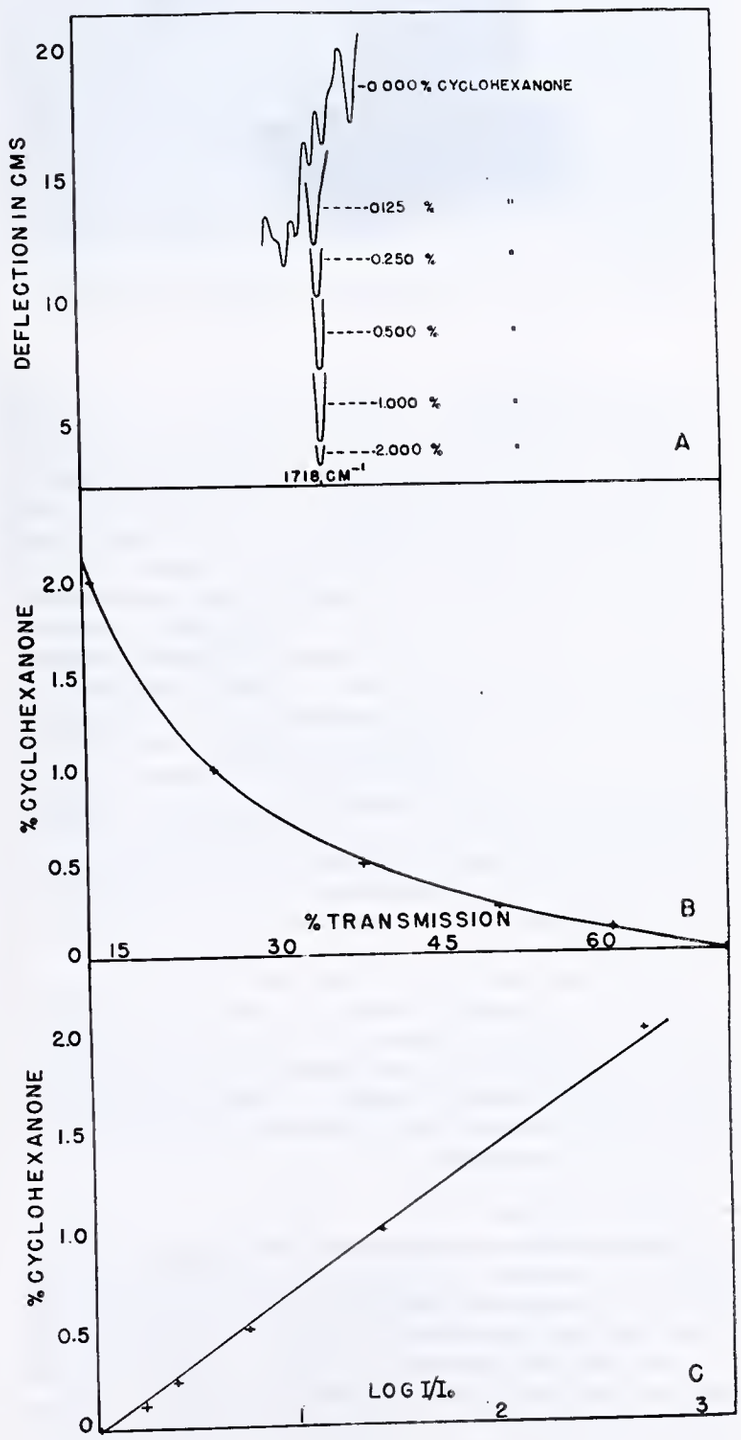


FIGURE 7. ONE COMPONENT INFRARED ANALYSIS FOR CYCLOHEXANONE IN CYCLOHEXANE

x = 0.2 mm.; spectral slit, ca. 8 cm.$^{-1}$

A. Automatic record comparison
B. Empirical working curve
C. Beer's law graph

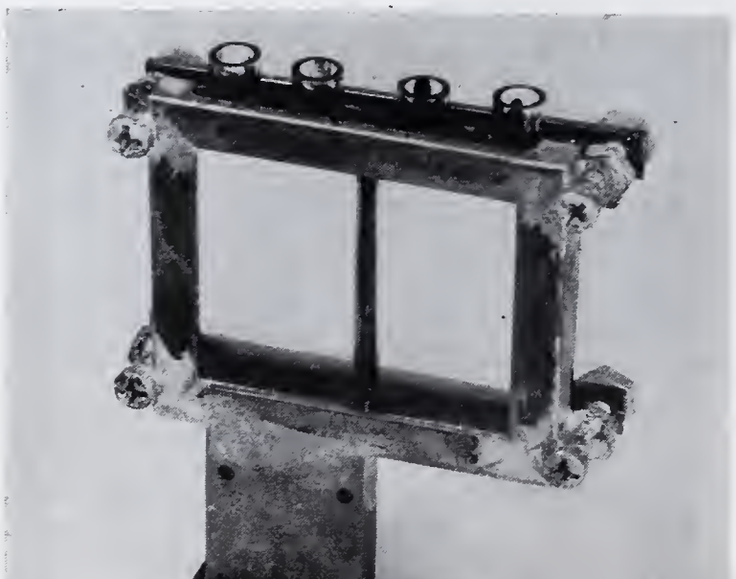


FIGURE 8. DOUBLE ABSORPTION CELL FOR SPECIAL ANALYSES

in a spectral region where considerable energy is available, for which the impurity does show greater absorption than the major component. The analysis is made with a double absorption cell, as shown in Figure 8, mounted in such a way that either side can be placed in the radiation path so quickly that very little change in thermocouple equilibrium occurs in the transition. By increasing the slit width and amplification factor a galvanometer deflection for $I_{0\nu}$ of 5 or 6 meters can be obtained. A standard material (usually the pure major component) is placed in one side of the double cell while the unknown is placed in the other. By shuttling the cell back and forth several times, an accurate value of the change in galvanometer spot reading from one cell to the other can be made. The unknown is then replaced by known standards and the method repeated until the unknown sample is bracketed with sufficient accuracy. It would not be possible to measure the 5- or 6-meter deflection of I_0 with great accuracy but if a change of 0.5 mm. between the two cells can be measured this would correspond to an accuracy in energy transmission of 1 part in 10,000.

In many cases, analyses can be made with surprising accuracy by means of such special methods. It must be remembered, however, that all types of one-frequency analyses depend entirely on the assumption that no other impurity is present which absorbs strongly at the given frequency. If this assumption is not valid it may be necessary to check the absorption of an impurity at two or three chosen points.

2. Single Major Component

In measuring a major component of a mixture any of the above methods may be used. An application of method *c* is shown in Figure 9. It was desired to measure the rate of polymerization of styrene under varying catalytic conditions. Monomeric styrene has absorption bands at 1628 cm.$^{-1}$ and 1412 cm.$^{-1}$ which are functions of the vinyl group. Since these bands disappear in the polymer, they may be used as unique measurements for the amount of monomer present. Using the per cent transmission at these frequencies of the starting material as 100 per cent concentration a value of k_{1628} and k_{1412} was obtained after making correction for scattered light and background absorption. These values of k were then used to determine the concentration of monomer in subsequent samples. Figure 9 shows a typical reac tion rate curve obtained on small samples extracted from th reaction kettle at given time intervals. The scattering of th points from a smooth curve indicates the accuracy to be ±1 pe cent of total composition.

II. DETERMINATION OF TWO COMPONENTS

1. Impurities

a. Independent Absorption Frequencies. In favorable case of analyzing for two or more impurities, the analytical bands ca sometimes be chosen so that, where one impurity has a strong ab sorption, the major component and the other impurity hav practically the same small absorption. An example is th analysis of butyl ether and butyl alcohol in butyl acrylate. Th working curve method was used as shown in Figure 10. The poin scattering shows an accuracy of ±0.5 per cent.

b. Dependent Absorption Frequencies. There may aris occasions where the favorable situation above does not hold. A example is the analysis of acrylonitrile for water and ethylen cyanhydrin. Each impurity has a hydroxyl absorption, water a 3430 cm.$^{-1}$ and cyanhydrin at 3350 cm.$^{-1}$ The analyses wer made on an instrument which had a negligible amount of scat tered light and the quartz absorption cell thickness was so ad justed that Beer's law would hold over the transmission rang used. The per cent transmissions of known standards were ob tained by dividing the sample transmission by that of pur acrylonitrile so that the resultant transmissions could be ascribe to the impurity alone. From these transmissions, values of k, were obtained for each impurity at each frequency. Unknowns were then analyzed by determining the log of the per cent transmission at each frequency and solving the two simultaneous equations for the concentrations. The accuracy of the analysis was ±0.5 per cent for cyanhydrin and slightly better than this for water.

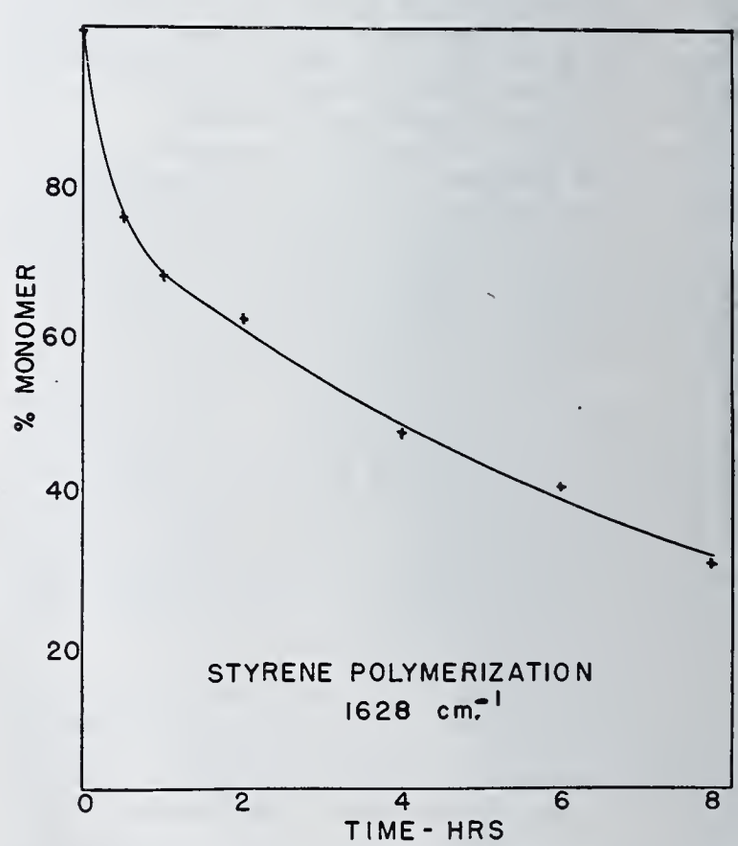


FIGURE 9. INFRARED MEASUREMENT OF A RATE OF STYRENE POLYMERIZATION

0.3 gm. sample in 10 cc. of CCl_4
x = 1 mm.
ν = 1628 cm.$^{-1}$
Spectral slit, ca. 9 cm.$^{-1}$

Two Major Components

For two major materials any of the above methods may be ed. In cases where the two bands chosen are unique and inde- ndent of other impurities, very rapid analysis can be made by ing deflection ratios instead of per cent transmissions. For ample, it may be desired to obtain the ratio of butane to iso- itane in an unknown mixture of these two materials. Isobutane as a strong band at 1175 cm.$^{-1}$ whereas butane is analyzed at 75 cm.$^{-1}$ An analysis can be made simply by obtaining gal- anometer deflections as a measure of the radiation passing rough the sample at each frequency. The ratio of the two de- ections can then be correlated with the ratio of the concentra- ons by means of a working curve previously obtained on known andards. The method is desirable in that it does not require a ll evacuation to determine I_0 nor is its accuracy sensitive to nall variations in the total gas pressure used.

III. DETERMINATION OF MULTICOMPONENTS

Impurities

There may be a case where the favorable situation of the butyl crylate impurities does not exist and yet it is not expedient to se Beer's law directly. A working curve method may be used by pplying the proper corrections to the graphs before interpreting he results.

A terpene is converted catalytically to *p*-cymene with the esultant impurities, *p*-menthane, dipentene, and dimethyl- tyrene. It is desired to analyze *p*-cymene for these three najor impurities. A comparison of k_ν's for the four pure ma- erials shows that *p*-menthane can be analyzed directly but hat the dipentene and the dimethylstyrene impurity absorp- ions must each be corrected for the amount of the other im- urities present.

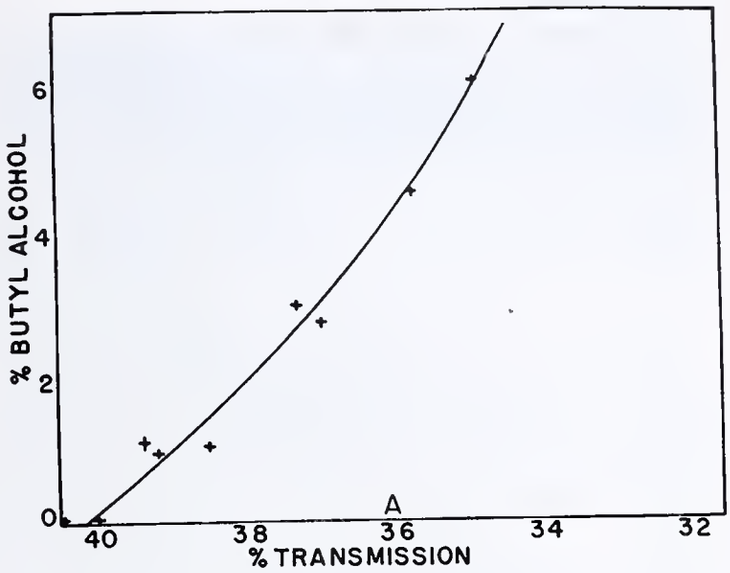
The working curves for this analysis are shown in Figure 11. Because a large number of samples was involved, it was expected hat there would be some change in transmission of the fixed ab- orption cell with use. Therefore, the working curves are plotted n terms of the difference in transmission at each chosen frequency etween pure *p*-cymene and the unknown mixture. By neasuring pure *p*-cymene at intervals throughout the analyses his change in cell absorption could be accounted for auto- natically.

The working curve for *p*-menthane, which had to be de- termined as the first step in the analysis, is shown in Figure 11,*A*. At the dimethylstyrene frequency, the absorption of *p*- menthane is less than that of *p*-cymene. Therefore, it was necessary to add the correction given in the caption of Figure 11,*B*. After this correction was made, the concentration of dimethylstyrene was determined. The standards used for this working graph were made up with varying concentrations of *p*-menthane in order to determine this correction. At the frequency of dipentene absorption both *p*-menthane and di- methylstyrene have absorptions which are greater than *p*- cymene and approximately equal. Therefore, the correct factor must be subtracted as indicated in the caption to Figure 11,*C*. In each case the point scattering of the standards shows an accuracy of the order of ±1 per cent.

In order to check the analysis, two standard mixtures were prepared and analyzed as unknowns. The results of these analyses are as follows:

	X_1, %		X_2, %	
	Actual	Infrared	Actual	Infrared
p-Menthane	10.7	10.0	7.5	6.5
Dimethylstyrene	7.1	7.0	5	4.5
Dipentene	3.6	4.0	2.5	2.5

This method of analysis has been extended to the case of measuring five impurities in the presence of a major component



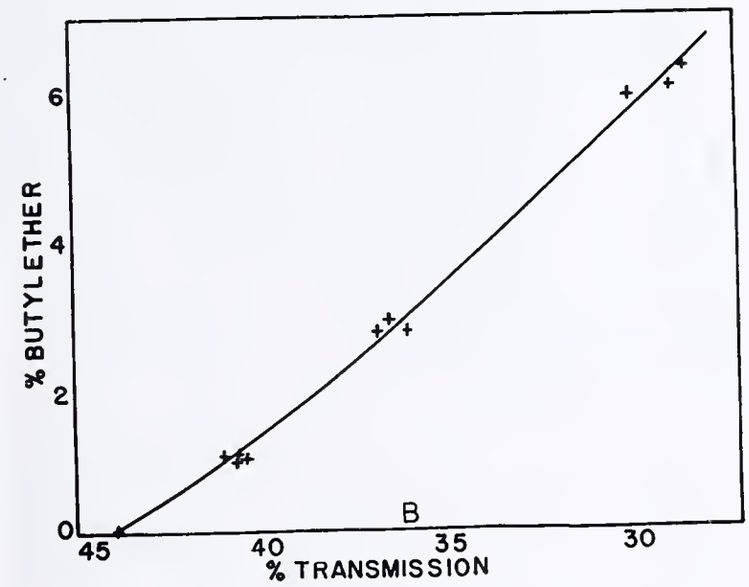


FIGURE 10. INFRARED ANALYSIS OF BUTYL ACRYLATE FOR THE IMPURITIES BUTYL ALCOHOL AND BUTYL ETHER

Sample diluted by volume one part to two parts CCl_4. x = 0.1 mm.

A. Butyl alcohol ν = 1030 cm.$^{-1}$; spectral slit, ca. 6 cm.$^{-1}$
B. Butyl ether ν = 1125 cm.$^{-1}$; spectral slit, ca. 6 cm.$^{-1}$

which is present to 60 or 70 per cent with the accuracy for each component being ±1 per cent of the total mixture. In one instance the catalytic reaction produced samples which were dis- colored by slight amounts of a dark, tarry residue. The spectro- scopic effect of these unknown materials on the analysis was allowed for by collecting some of the residue under careful dis- tillation and adding it to the standards in amounts necessary to match the visible light absorption of the unknowns. The method is rough and detracts from the absolute accuracy of the known component analysis, but it still allows for good relative measure- ments for testing the effects of varying catalytic conditions.

While performing these analyses on a large number of un- knowns, it was found advisable to check the standards at fre- quent intervals. Once the working graphs were determined, the time of analysis for each unknown, including standard mixture calibration, sampling, and computation was about 0.5 hour in the sense that fifteen analyses could be made in an 8-hour day.

2. Major Components

The choice of the best analytical method for a mixture comprising several major components depends to a great extent on the variations in concentration which are encountered. If these variations are small, as would be the case in production batches, the working graph method of the above section is quick and easy.

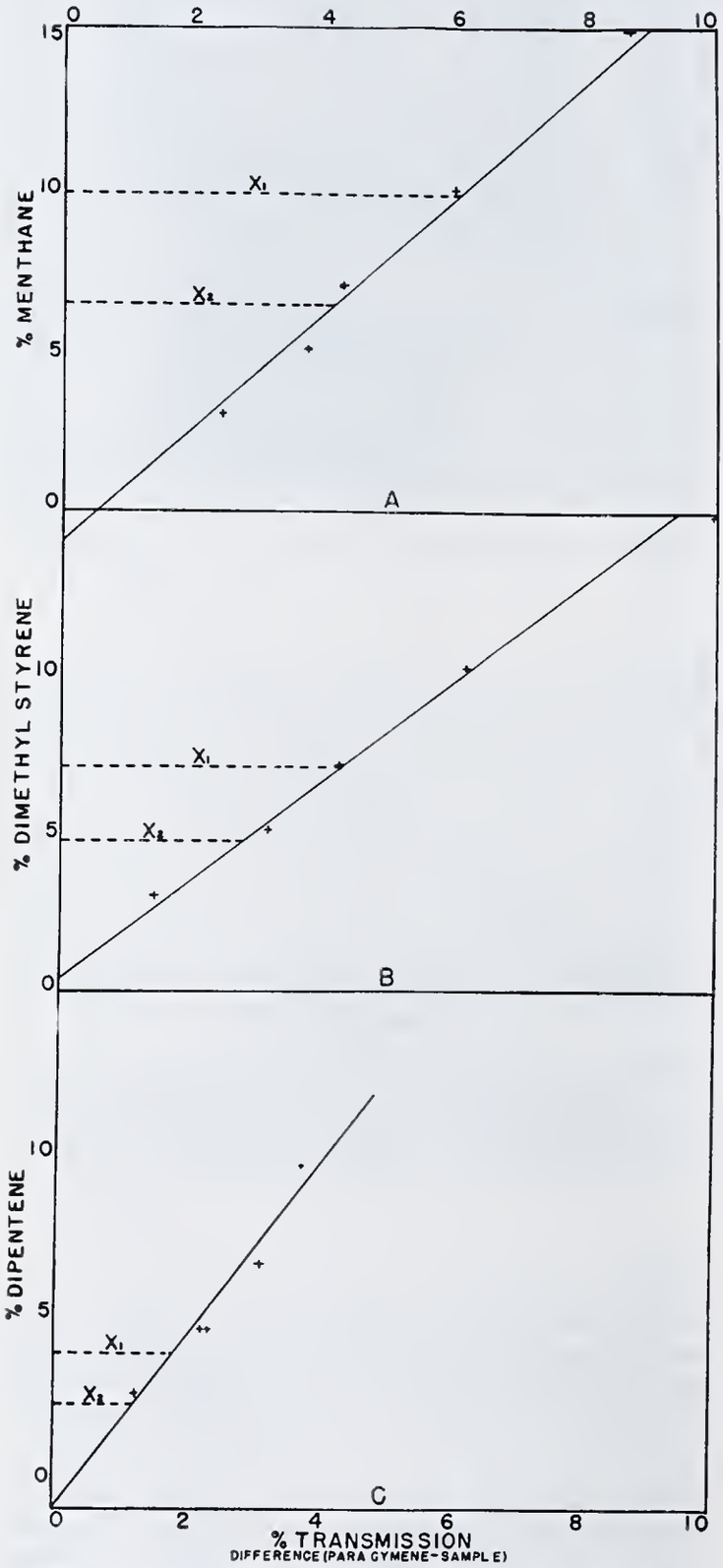


FIGURE 11. INFRARED ANALYSIS OF *p*-CYMENE FOR *p*-MENTHANE, DIMETHYLSTYRENE, AND DIPENTENE

x = 0.1 mm.

A. Working curve for *p*-menthane
ν = 1177 cm.$^{-1}$; spectral slit, ca. 6 cm.$^{-1}$

B. Working curve for dimethylstyrene
ν = 1570 cm.$^{-1}$; spectral slit, ca. 9 cm.$^{-1}$
Correction: For every 5 per cent of *p*-menthane present, add 0.7 per cent to difference percentage before plotting.

C. Working curve for dipentene
ν = 963 cm.$^{-1}$; spectral slit, ca. 5 cm.$^{-1}$
Correction: For every 5 per cent of total *p*-menthane plus dimethylstyrene, subtract 0.6 per cent from difference percentage before plotting.

Over small ranges of concentration the working graphs w approximate straight lines. In such cases changes in absorptic cell condition can be checked at intervals and a point determin through which the straight line of known slope may be draw This method has been applied to mixtures of substituted anilin and the results obtained agreed within ±1 per cent with di tillation analyses on the same samples. However, if the conce trations vary over a wide range, a direct calculational applic tion of Beer's law would be accurate but time-consuming, while method of working graphs on semilog paper would be fast wit some sacrifice in accuracy.

PRESENT STATUS OF INFRARED SPECTROSCOPY IN INDUSTRY

The initial phase of infrared spectroscopy in industry was ex ploratory in nature. It has been known for many years that suc correlations as those described in the section on Qualitativ Analysis could be made between observed spectral characteristic and the molecular structure of the material being studied. Th fact that an infrared absorption spectrum is a unique char acteristic of a compound had suggested that this spectral regio could be useful for identification and analysis of unknown ma terials and mixtures. Starting with this basic knowledge, spectros copists in those companies which have shown an interest in th field have had to construct their own spectrometers, build up reference library of absorption spectra of pure materials, establis structural correlations, and develop their own methods of quanti tative analysis. Not only has this fundamental work been done but also sufficient preliminary applications and results have bee derived to prove conclusively that infrared pays its own way and is rapidly becoming a practical necessity to any research or pro- duction organization handling organic chemicals.

Industrial infrared spectroscopy is now entering its second phase—the development from proving potentialities under ideal research conditions to widespread applications under any prac- tical conditions which might occur. This expansion does not imply that all efforts must be bent toward making infrared techniques routine but it does imply that the basis upon which infrared research is conducted has changed. It has passed the stage of hiring a trained infrared spectroscopist with the general instruction that he should build an instrument and then proceed to demonstrate its utility. Neither sufficient time nor trained personnel is available to permit such an approach. With the large number of companies now interested in conducting infrared re- search, it is necessary that commercial spectrometers be available, that there be sufficient background information in the literature so that an operator may be put on one of these instruments to produce results immediately without having to spend a long time acquiring basic data.

The first person to recognize this demand was the infrared spectroscopist already at work in the field. He was called upon to study the possibility of performing analyses which were difficult or tedious by chemical methods. Once a satisfactory analysis had been established, his research instrument was generally tied up for a month or more on routine work while re- search on further exploratory problems suffered. For his own protection, the spectroscopist has had to develop simple, compact instruments having sufficient resolving power to handle the majority of the analytical problems which might be encountered. With such instruments available, the role of the research spec- trometer in analysis may be restricted to obtaining the pure spectra of the components involved. From these spectra the analytical frequencies can be chosen and the rest of the analysis performed by means of the small spectrometer. For convenience in setting to these frequencies, mechanical stops are provided. These small instruments can be operated in the infrared labora- tory or moved to the site of the chemical reaction if operating conditions are favorable.

In accordance with the mutual desire of the industrial spectroscopist and the instrument maker, these small instruments are being made commercially available. The manufacturer is always anxious to produce an instrument which has been designed, proved practical, and has a growing market. The research spectroscopist does not, in general, desire to carry out analyses on production samples once the details of analysis have been worked out. He prefers to refer the production man to a commercial source of instruments which will answer the requirements. Therefore, as far as small routine instruments are concerned, favorable progress is being made.

Unfortunately, this does not mean that the spectroscopist can turn back happily to problems intended for his research spectrometer. There are at least two important advances in the instrumentology of research spectrometers which must be made before the spectroscopist can devote himself entirely to the results obtained and not have to worry about the means of producing them. In the first place, most infrared spectra are obtained today as records of frequency *vs.* galvanometer deflection superimposed upon the radiation background. To obtain full significance from these results it is necessary to convert them to frequency *vs.* per cent transmission or log of per cent transmission. This conversion as it is now done involves considerable tedious labor and it is highly desirable that the instrument make this conversion automatically. In short, infrared needs a spectrophotometer to replace its present spectrometer. In the second place, most infrared recording is done by means of a very sensitive galvanometer amplifier. Unless the stability conditions of the building in which the instrument is used are favorable, such a mechanically sensitive device is not satisfactory. Certainly, under the conditions which exist in the average laboratory or plant, an improved detecting system is required. Therefore, it is highly desirable that a means of electronic amplification of thermocouple output be devised to replace the present use of galvanometers. There is a growing tendency to use pen recorders rather than photographic methods for recording the galvanometer deflections. This means that an electronic stage must be interposed between the galvanometer beam and the actual record. If the entire amplification from the radiation detector to the record could be done electronically, the use of some type of a split beam instrument would answer the instrumental problems of the infrared spectroscopist.

It will be a natural step for the manufacturer to go from the small routine spectrometer to the research type of instrument and it is to be hoped that the combination of instrument maker and spectroscopist will solve these problems in the near future. It will then remain for the spectroscopist to refine his analytical techniques and to broaden his knowledge and application of structural correlations, in order that infrared spectroscopy can make its maximum contribution to the advance of science.

LIBRARY OF REFERENCE CURVES

As shown above, it is very desirable to have available for reference purposes the per cent transmission curves for as many organic compounds as possible. These curves serve a threefold purpose. They may be used for studying correlations between molecular structure and spectral characteristics, for identification of unknown materials, and for determining in advance the possibilities of qualitative and quantitative infrared analysis of mixtures.

In presenting these spectra the following comments and cautions are offered:

It can readily be seen from inspection of the graphs that the spectra were not obtained primarily for purposes of publication. Since they were all obtained in connection with some particular problem, in a few cases, the sample thickness used or the spectral region studied renders the graphs incomplete. Although of limited usefulness, these curves are included. So far as possible pure materials were used, but occasionally the nature of the problem at hand and the pressure of work did not permit the use of exceptional care in this respect.

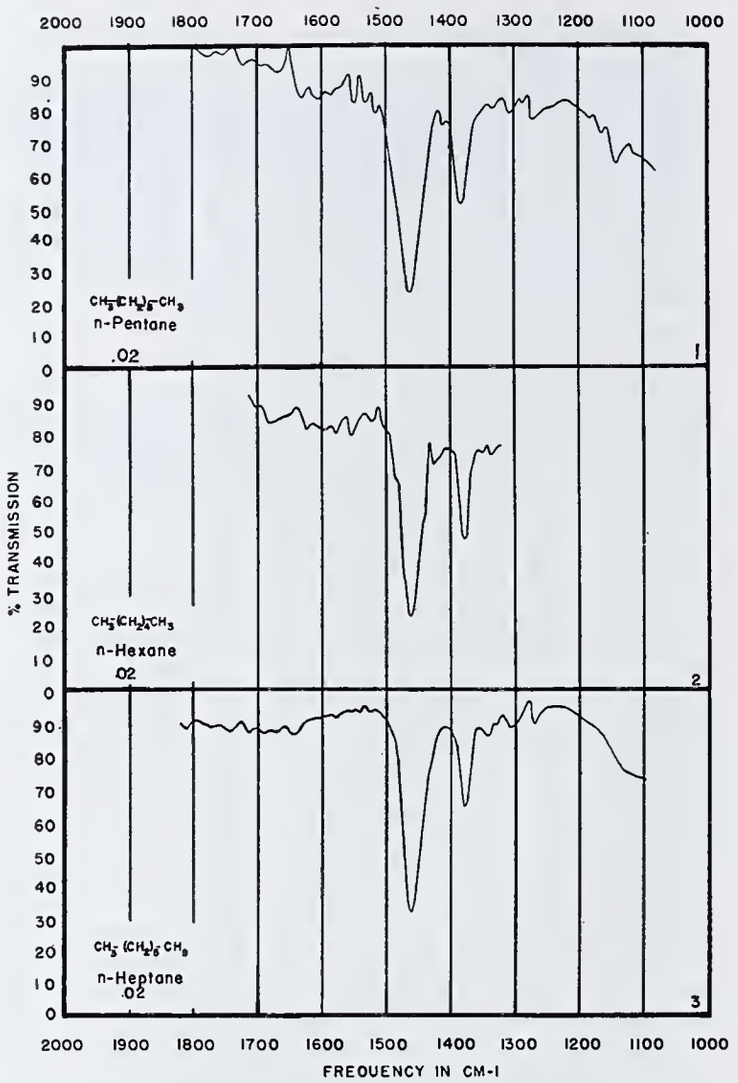
All the spectra shown were obtained on the instrument decribed in the section on Experimental Equipment and Technique, but over a period of years there have been changes in both the optical system and the sensitivity of the spectrometer. The spectral region above 2000 cm.$^{-1}$ has not been plotted (and in many cases not studied) because the poor dispersion of rock salt in this region limits the value of such studies.

The spectra shown from 2000 cm.$^{-1}$ to 750 cm.$^{-1}$ were made with an average spectral slit width of about 8 cm.$^{-1}$ in the 1600 cm.$^{-1}$ region grading down to about 5 cm.$^{-1}$ in the 1000 cm.$^{-1}$ region. The spectral slit widths for the plots between 2000 cm.$^{-1}$ and 1000 cm.$^{-1}$ were slightly greater than this. In each case the average accuracy of the frequency value of the absorption maxima is ± 5 cm.$^{-1}$, depending upon the time intervals between instrument calibrations. The values of per cent absorption have relative accuracies only, since, in plotting, allowance was not always made for scattered light and the energy transmission curve was not taken with every sample. The per cent readings of the bands should be considered only in the light of weak, medium, or strong absorption. All sample thicknesses for liquids are given by the cell spacer thickness in mm. or as capillary (cap.) where no spacer was used. If the material was run in vapor form, it is designated by (V), and in all such cases a 10-cm. absorption cell was used. These pressures were not measured, but were adjusted arbitrarily to give the desired degree of transmission.

The spectra are indexed in two ways: (1) an empirical formula index, and (2) an alphabetical index following the form used in *Chemical Abstracts*, with cross references wherever the name on the graph differs radically from *Chemical Abstracts*. The curves are numbered serially and each index is made up in terms of these serial numbers. The order of presentation of the spectra is roughly according to the following organic characteristics:

A. Hydrocarbons
   1. Aliphatic
      a. Saturated
      b. Unsaturated
   2. Aromatic
B. Alcohols
   1. Aliphatic
   2. Aromatic
C. Ethers
   1. Aliphatic
   2. Aromatic
D. Carbonyl Compounds
   1. Anhydrides
   2. Esters
   3. Aldehydes
   4. Ketones
   5. Acids
E. Nitrogen Compounds
   1. Amines
      a. Aliphatic
      b. Aromatic
   2. Amides
   3. Nitriles
   4. Nitro
   5. Miscellaneous
F. Terpenes
G. Organic Chlorides
H. Miscellaneous

[On the following pages, 676 to 709, will be found 363 graphs along with an Empirical Formula Index and an Alphabetical Index.]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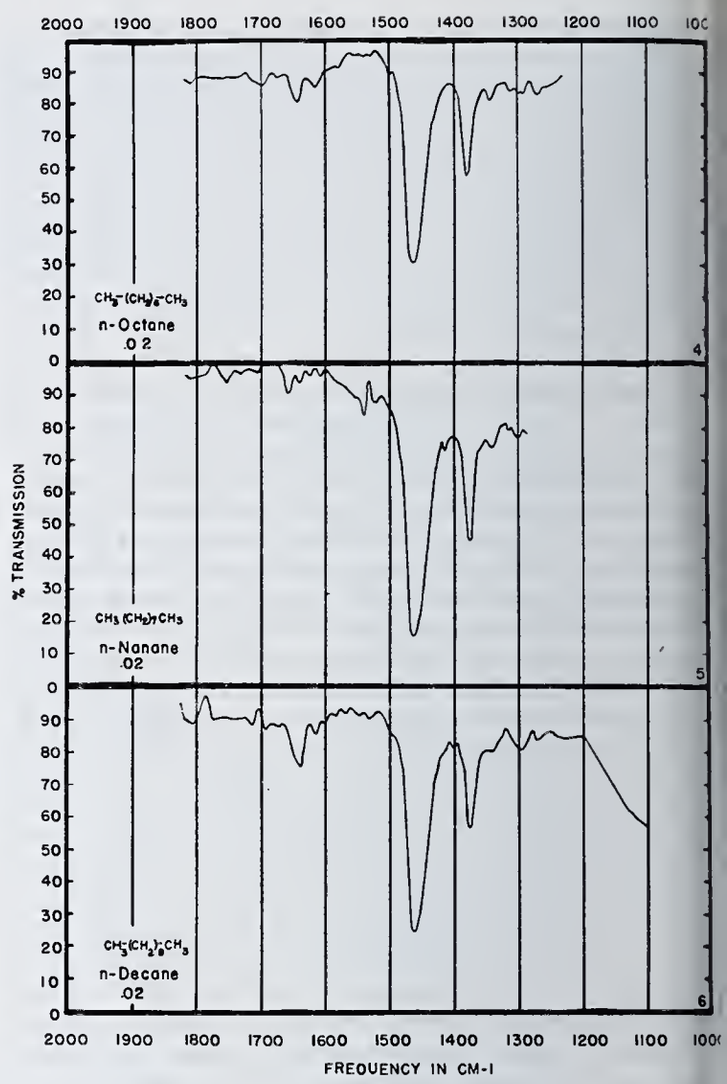
n-Hexane .02
n-Heptane .02


% TRANSMISSION
FREQUENCY IN CM-1
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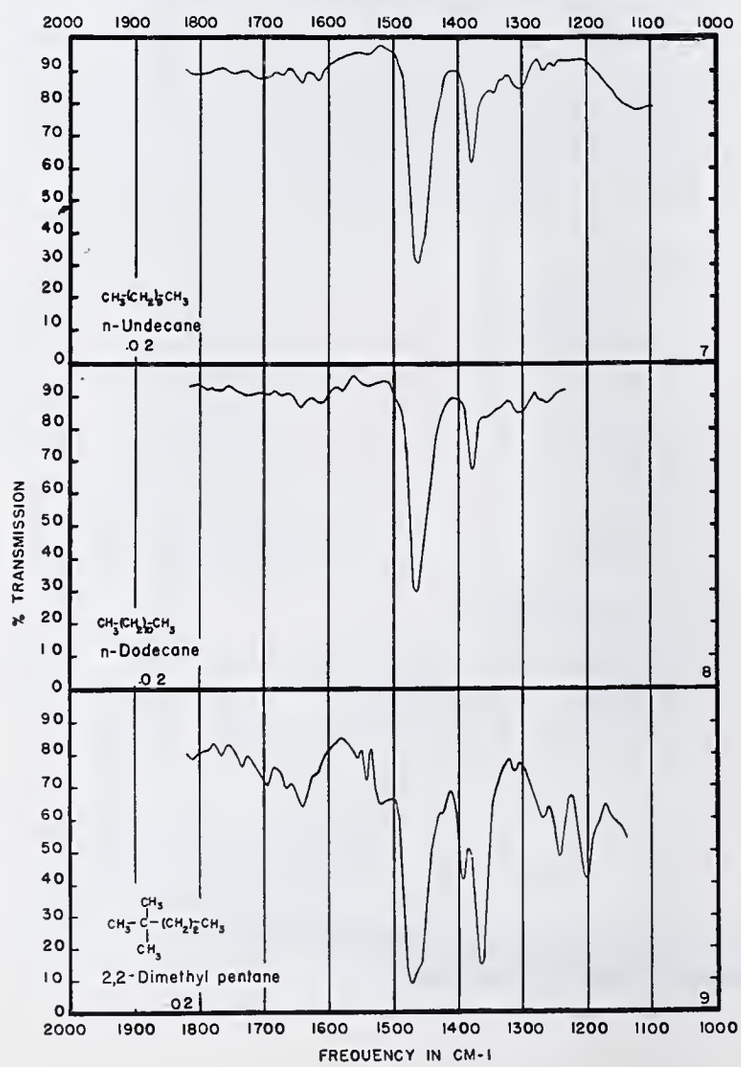
n-Nonane .02
n-Decane .02


% TRANSMISSION
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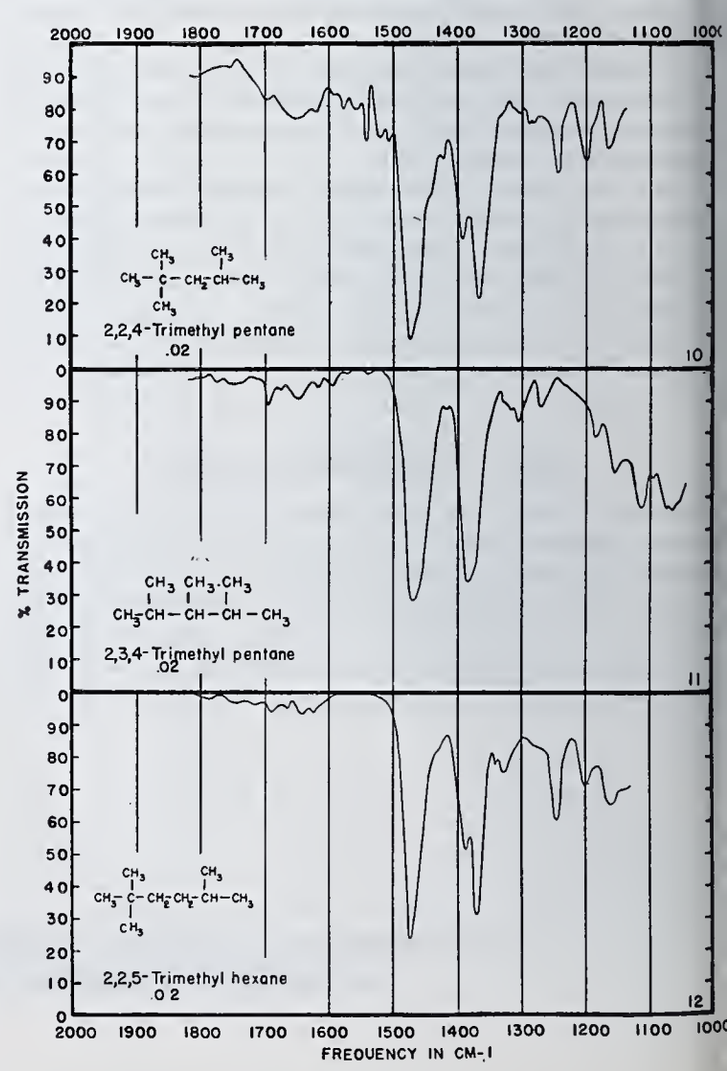
n-Dodecane .02
2,2-Dimethyl pentane .02


% TRANSMISSION
FREQUENCY IN CM-1
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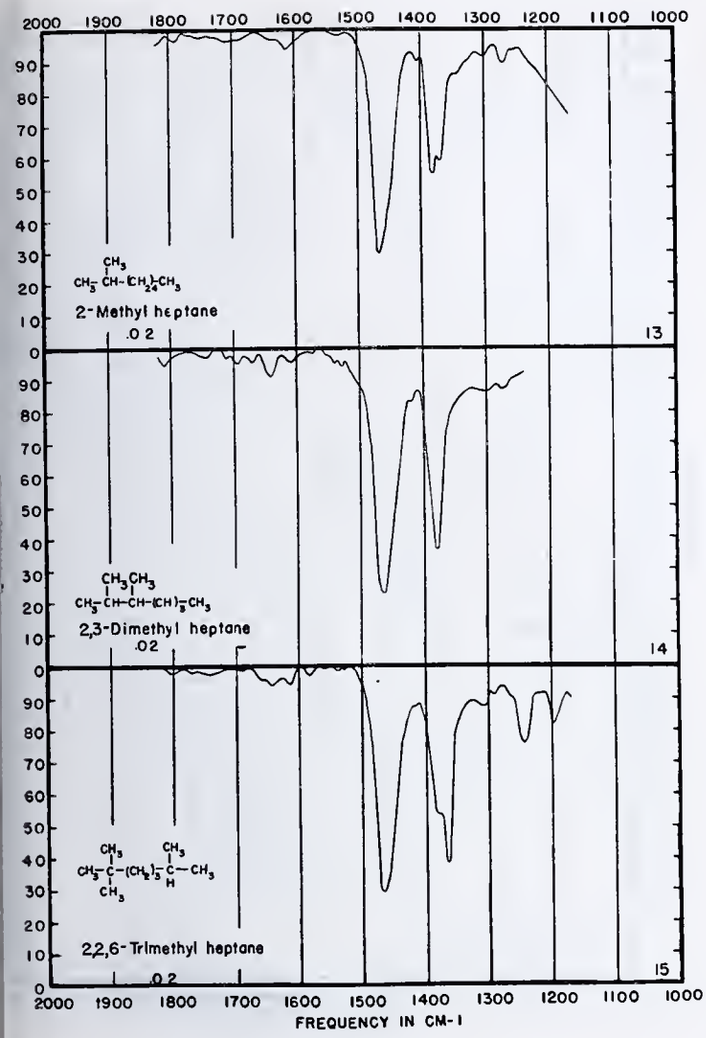
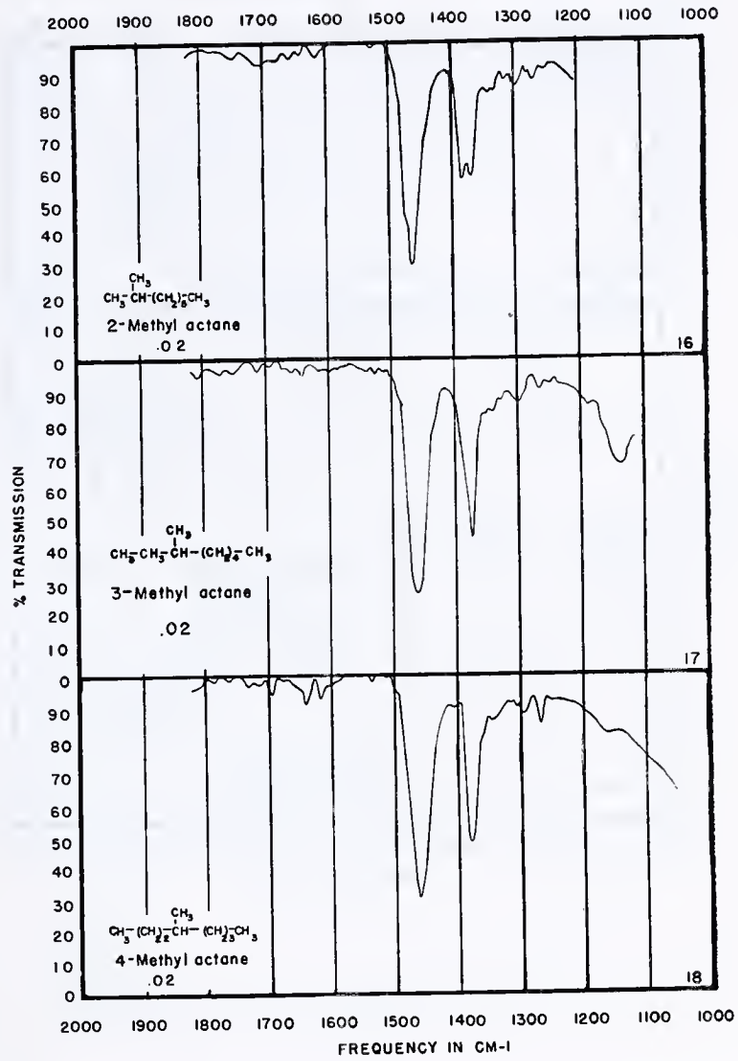
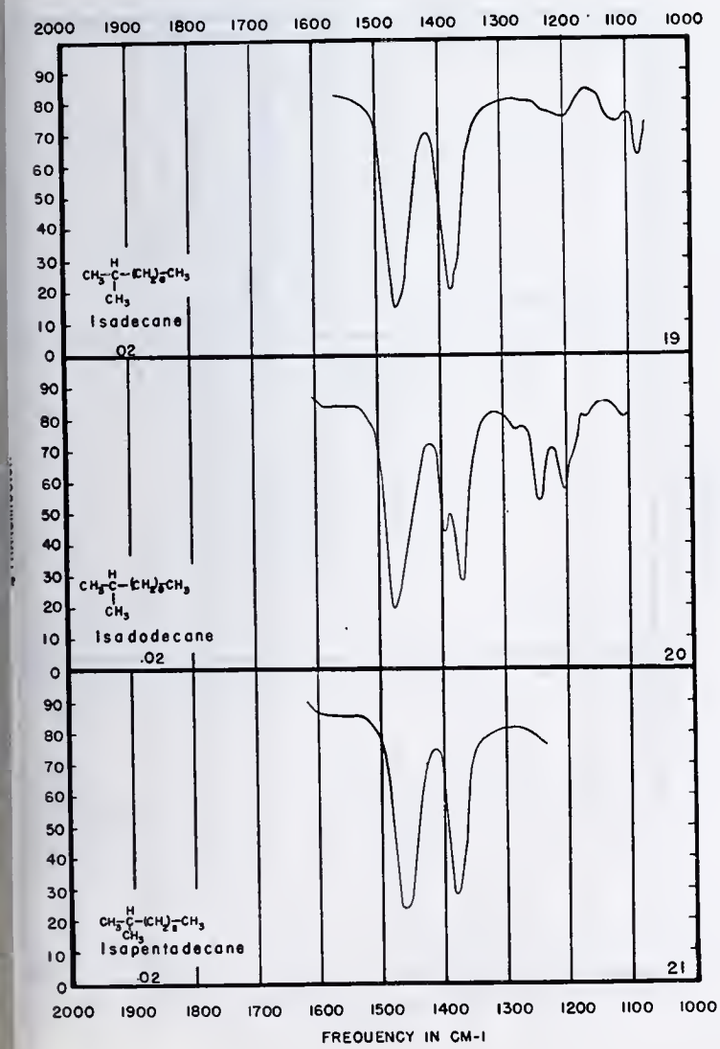
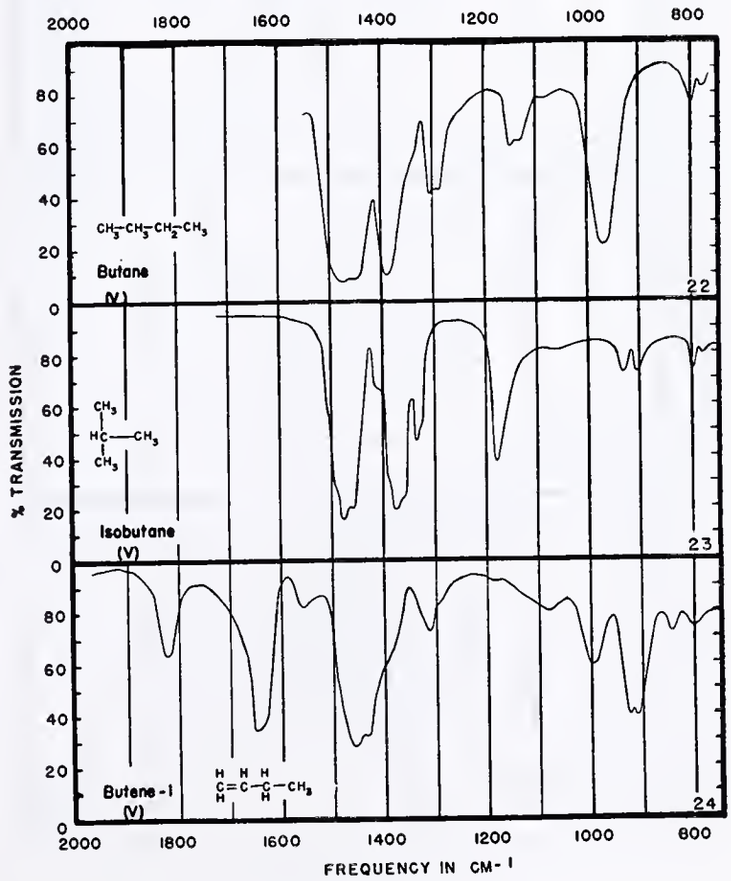
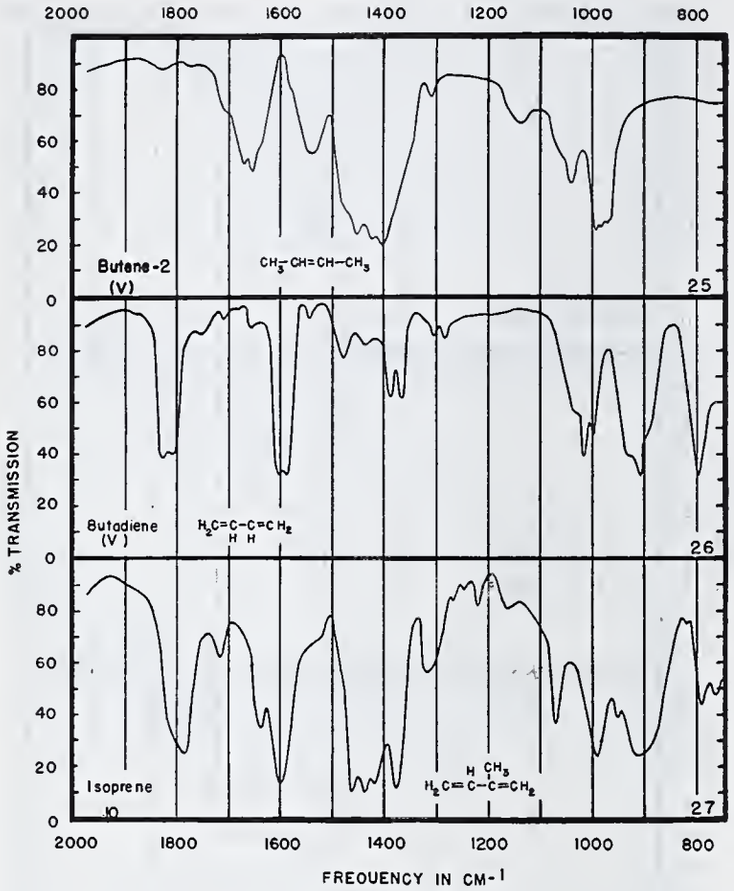
2,3,4-Trimethyl pentane .02
2,2,5-Trimethyl hexane .02

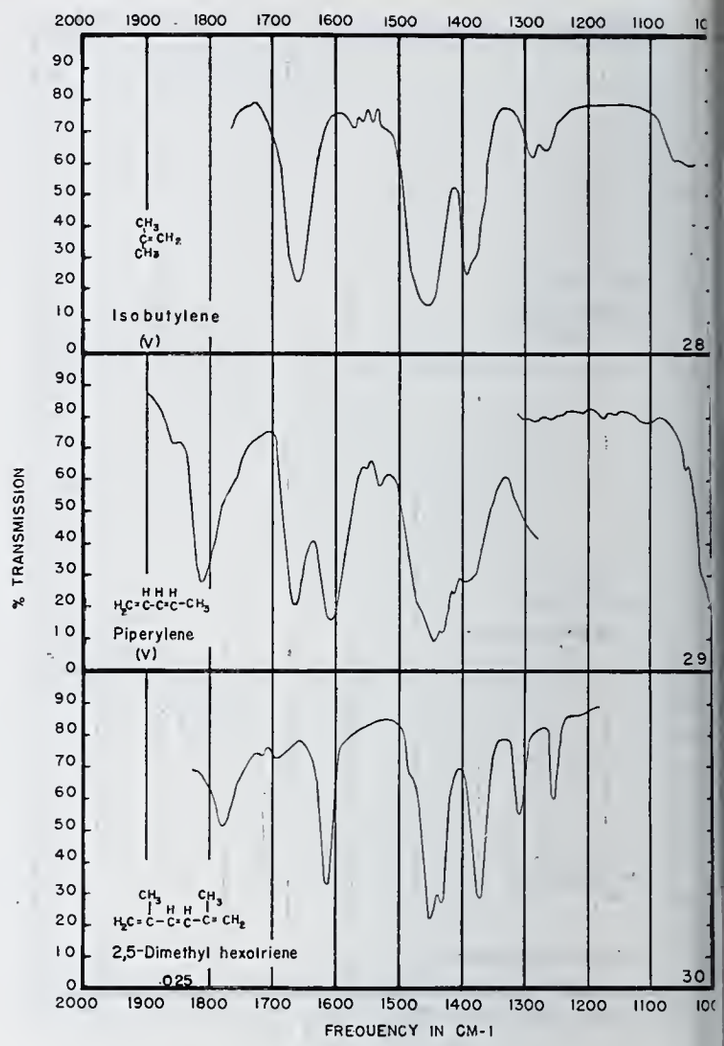

2-Methyl heptane
.02
13
2,3-Dimethyl heptane
.02
14
2,2,6-Trimethyl heptane
.02
15
FREQUENCY IN CM-1


% TRANSMISSION
2-Methyl octane
.02
16
3-Methyl octane
.02
17
4-Methyl octane
.02
18
FREQUENCY IN CM-1


Isodecane
.02
19
Isododecane
.02
20
Isopentadecane
.02
21
FREQUENCY IN CM-1


% TRANSMISSION
Butane
(V)
22
Isobutane
(V)
23
Butene-1
(V)
24
FREQUENCY IN CM-1


Butene-2
(V)
$CH_3-CH=CH-CH_3$
25
Butadiene
(V)
$H_2C=C-C=CH_2$
H H
26
Isoprene
.10
$H_2C=C-C=CH_2$
27
% TRANSMISSION
FREQUENCY IN CM-1


Isobutylene
(V)
28
Piperylene
(V)
29
2,5-Dimethyl hexotriene
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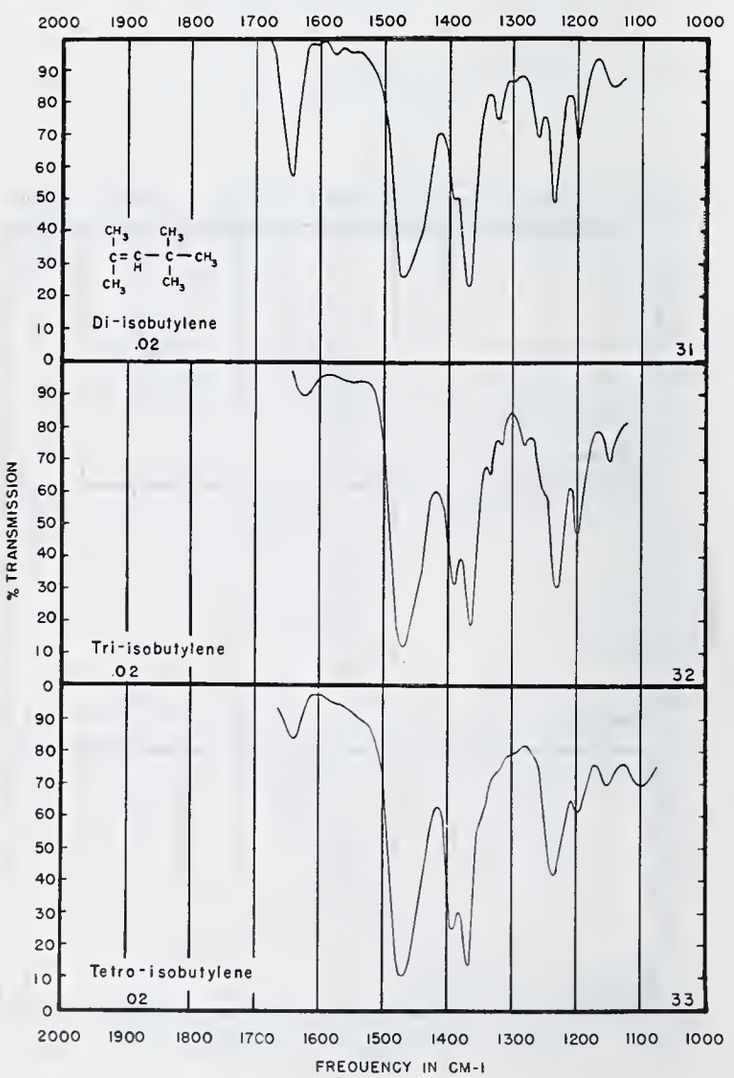
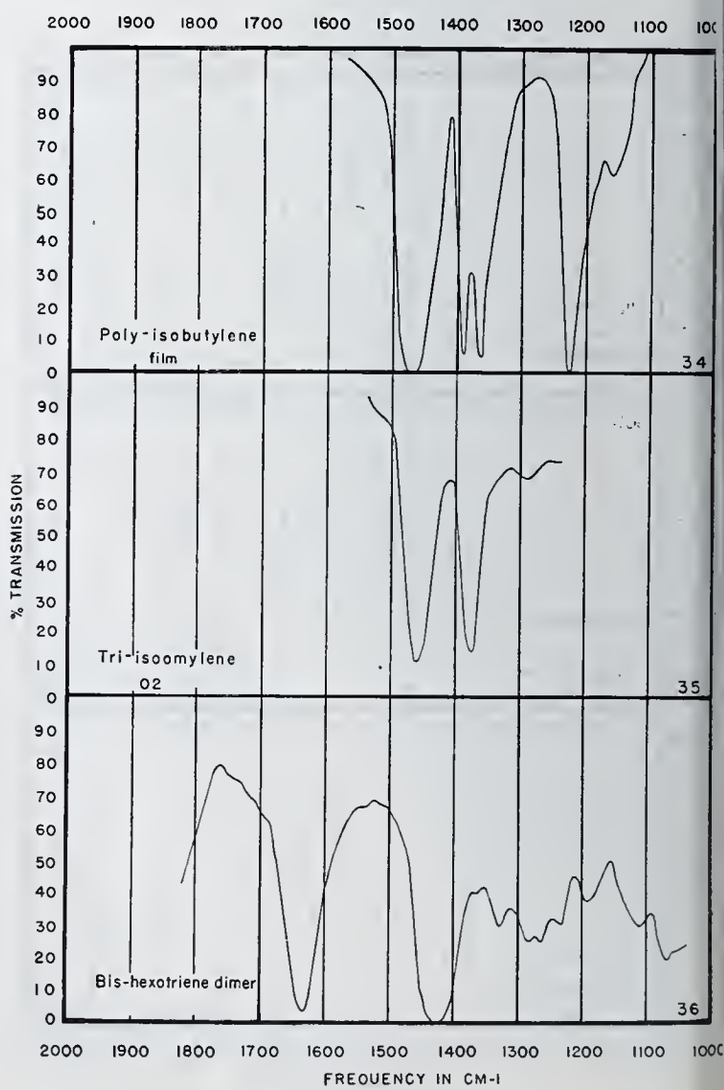
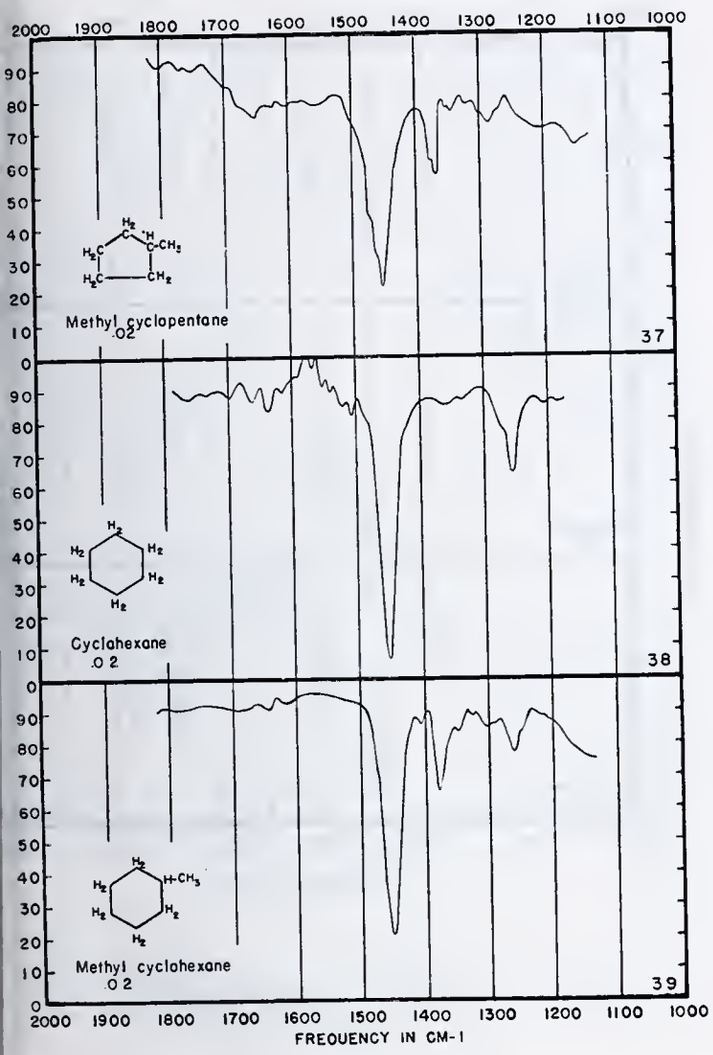
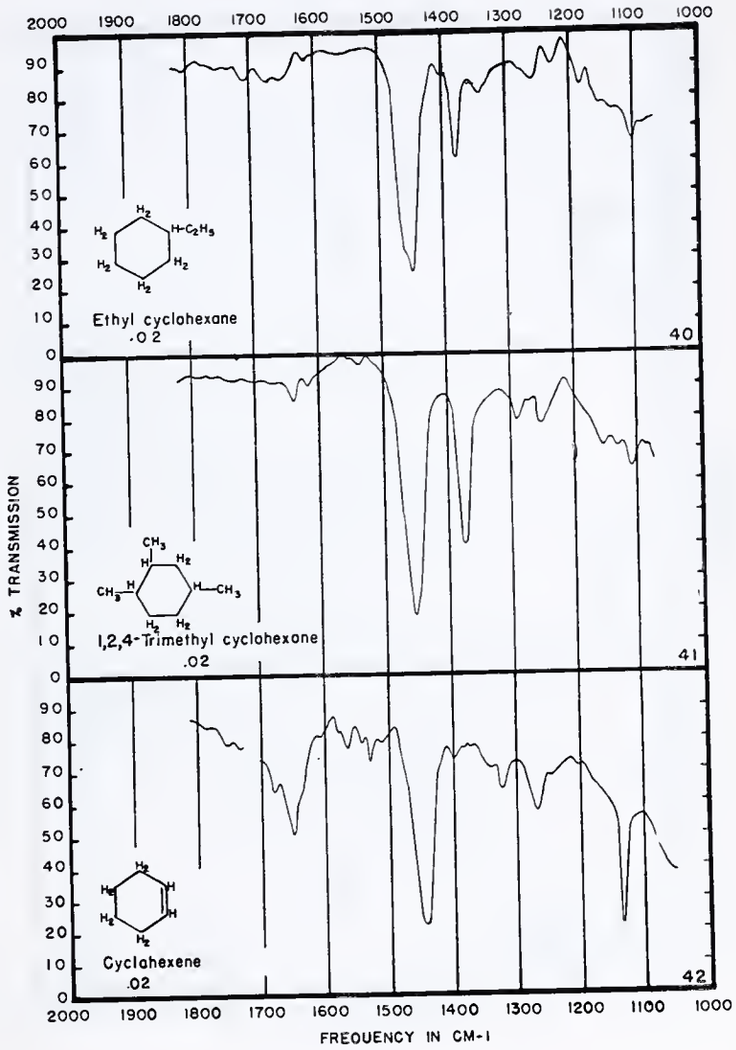
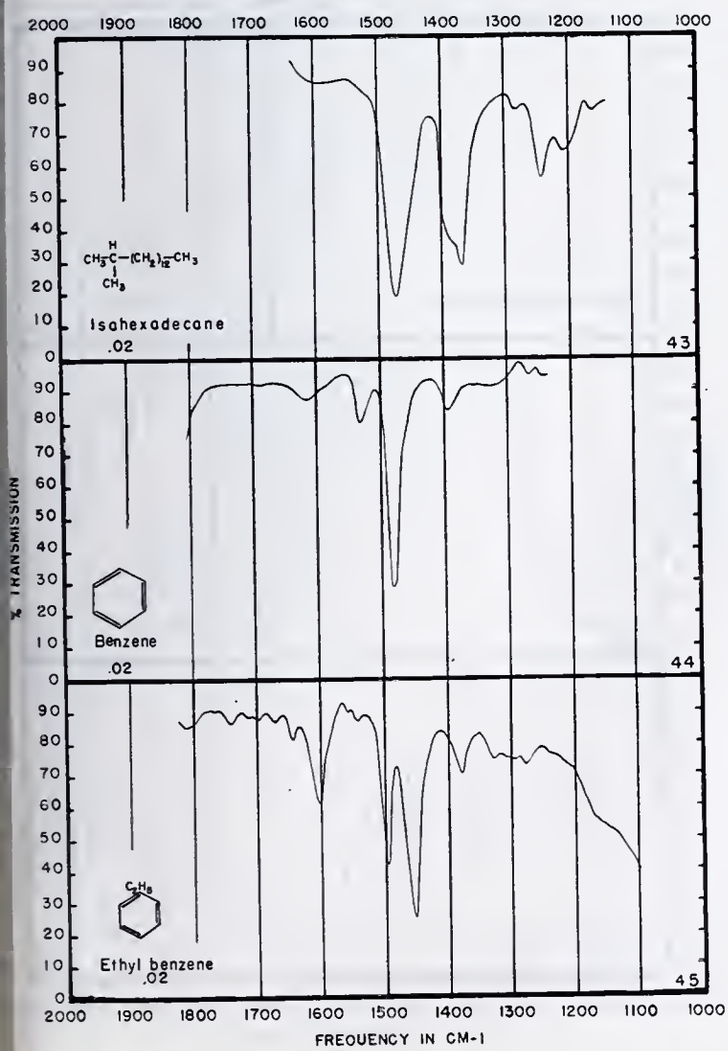
30
% TRANSMISSION
FREQUENCY IN CM-1


Di-isobutylene
.02
31
Tri-isobutylene
.02
32
Tetro-isobutylene
02
33
% TRANSMISSION
FREQUENCY IN CM-1


Poly-isobutylene
film
34
Tri-isoomylene
02
35
Bis-hexotriene dimer
36
% TRANSMISSION
FREQUENCY IN CM-1


Methyl cyclopentane
.02
37
Cyclohexane
.02
38
Methyl cyclohexane
.02
39
FREQUENCY IN CM-1


% TRANSMISSION
Ethyl cyclohexane
.02
40
1,2,4-Trimethyl cyclohexane
.02
41
Cyclohexene
.02
42
FREQUENCY IN CM-1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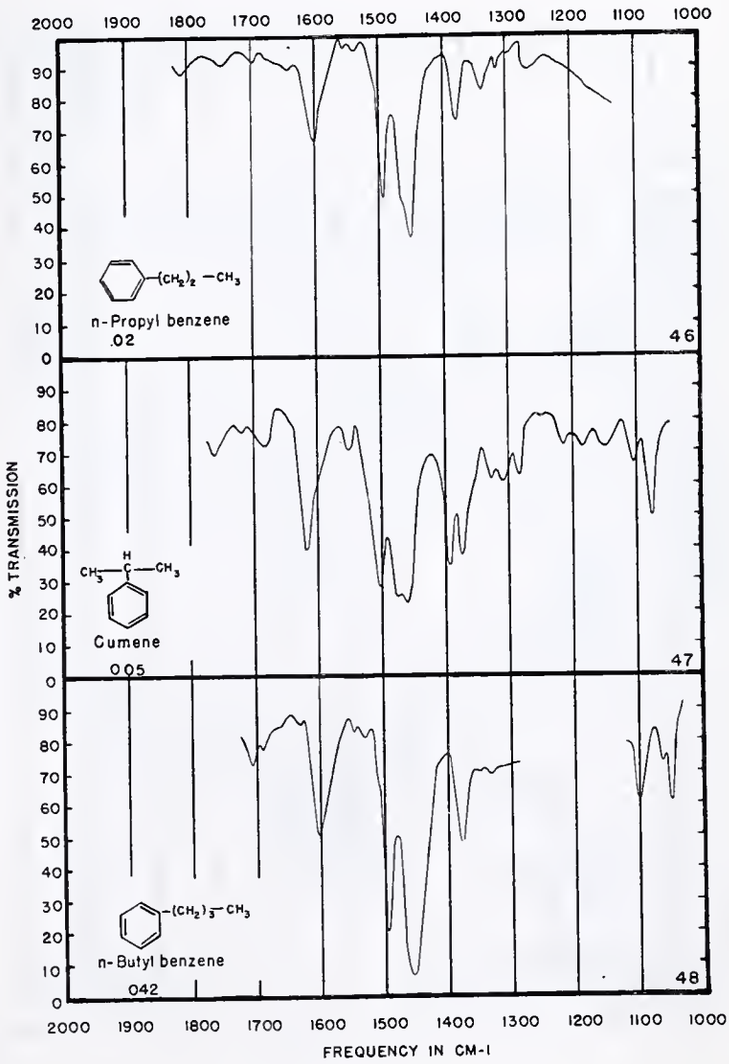
Benzene
.02
44
Ethyl benzene
.02
45
FREQUENCY IN CM-1


% TRANSMISSION
n-Propyl benzene
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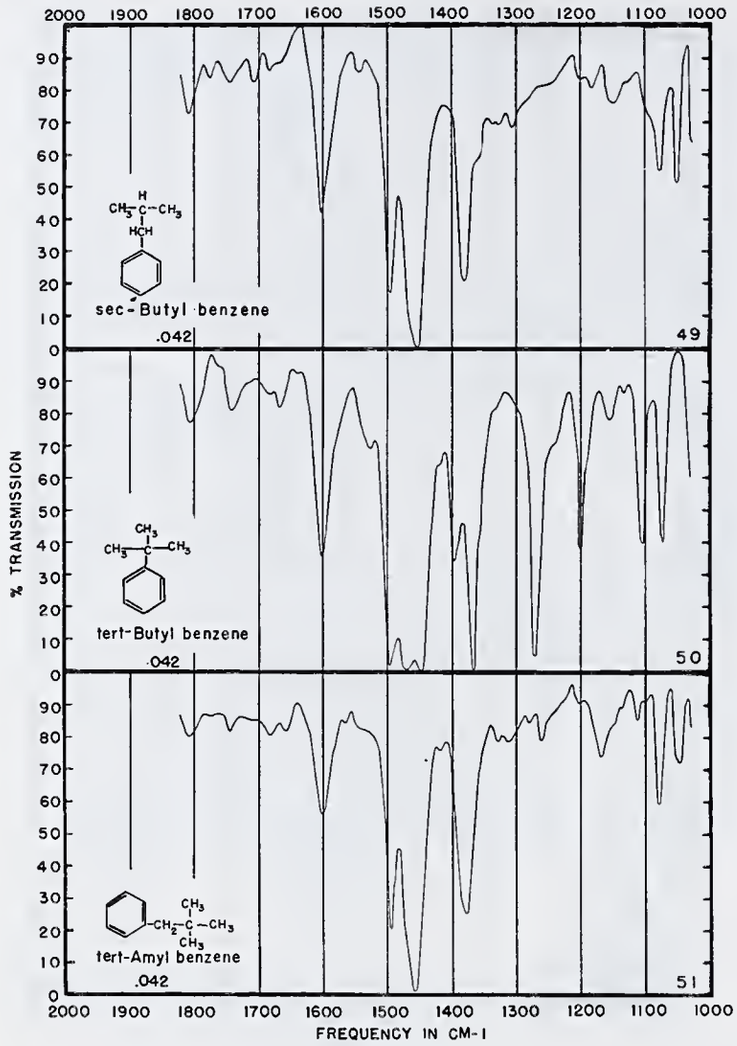
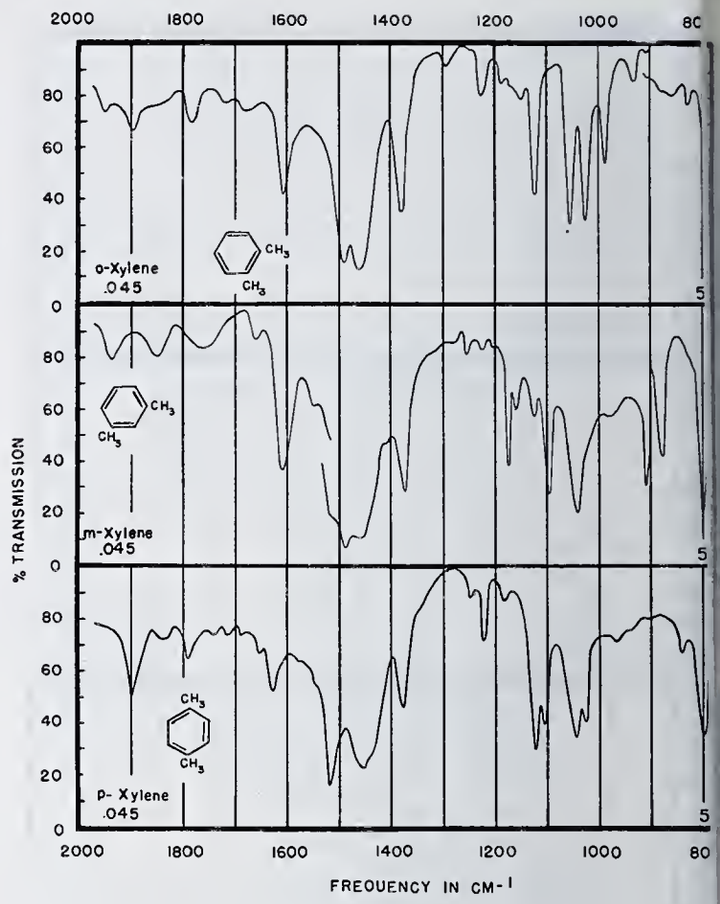
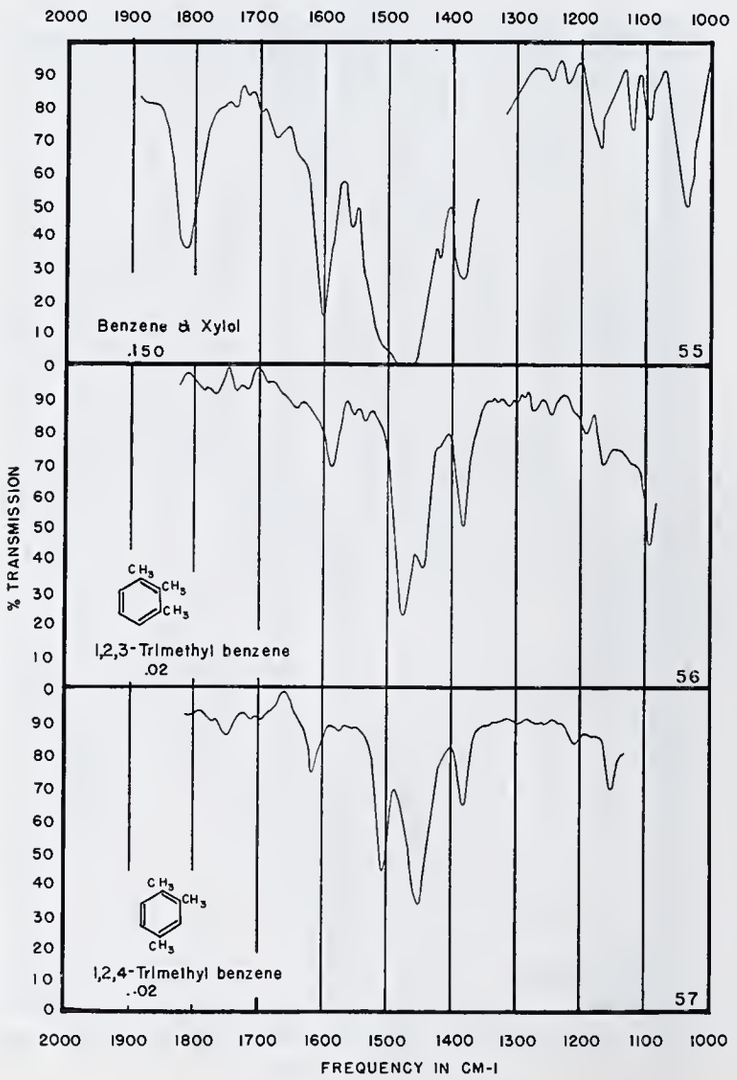
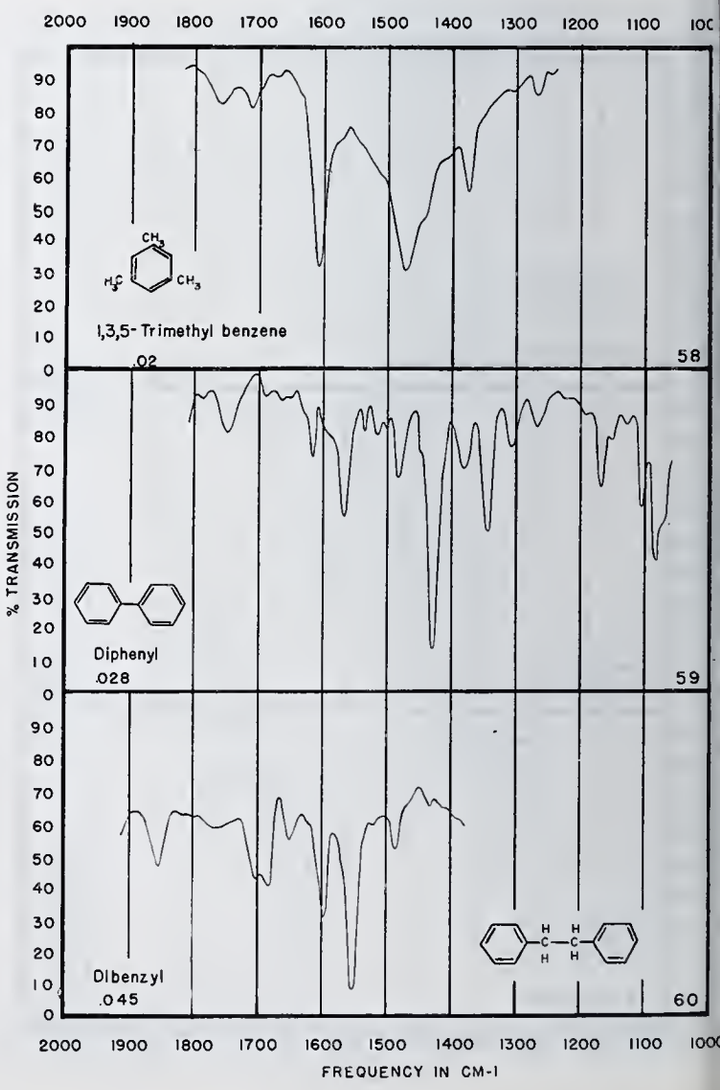
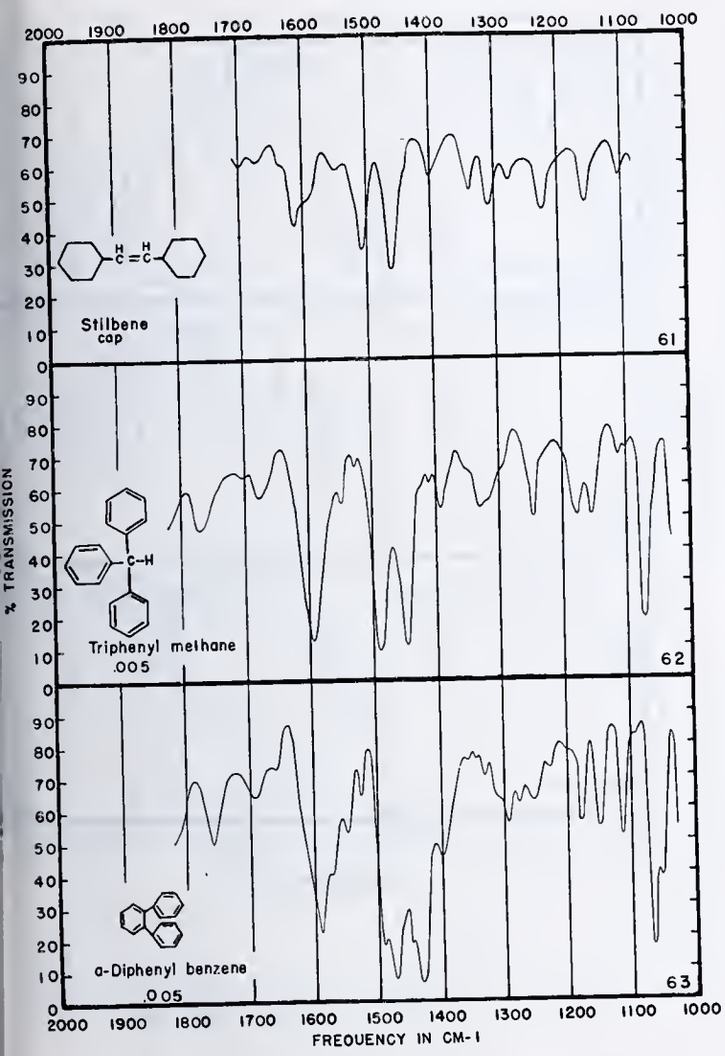
46
Cumene
005
47
n-Butyl benzene
042
48
FREQUENCY IN CM-1

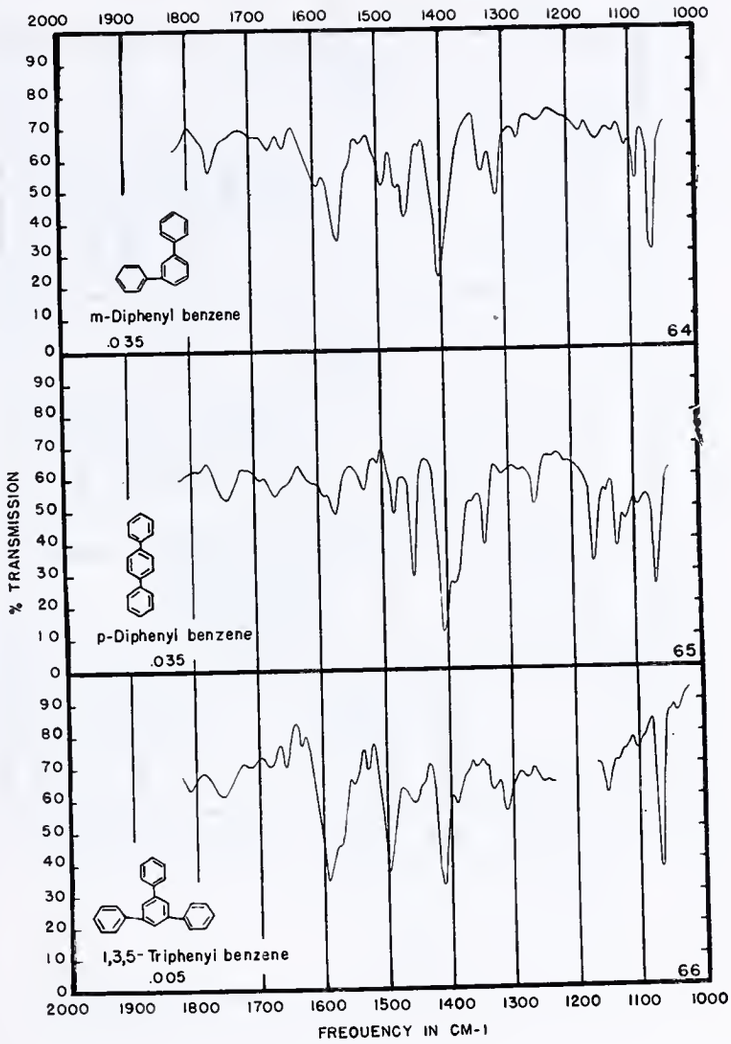

sec-Butyl benzene
.042
49
tert-Butyl benzene
.042
50
tert-Amyl benzene
.042
51
% TRANSMISSION
FREQUENCY IN CM-1

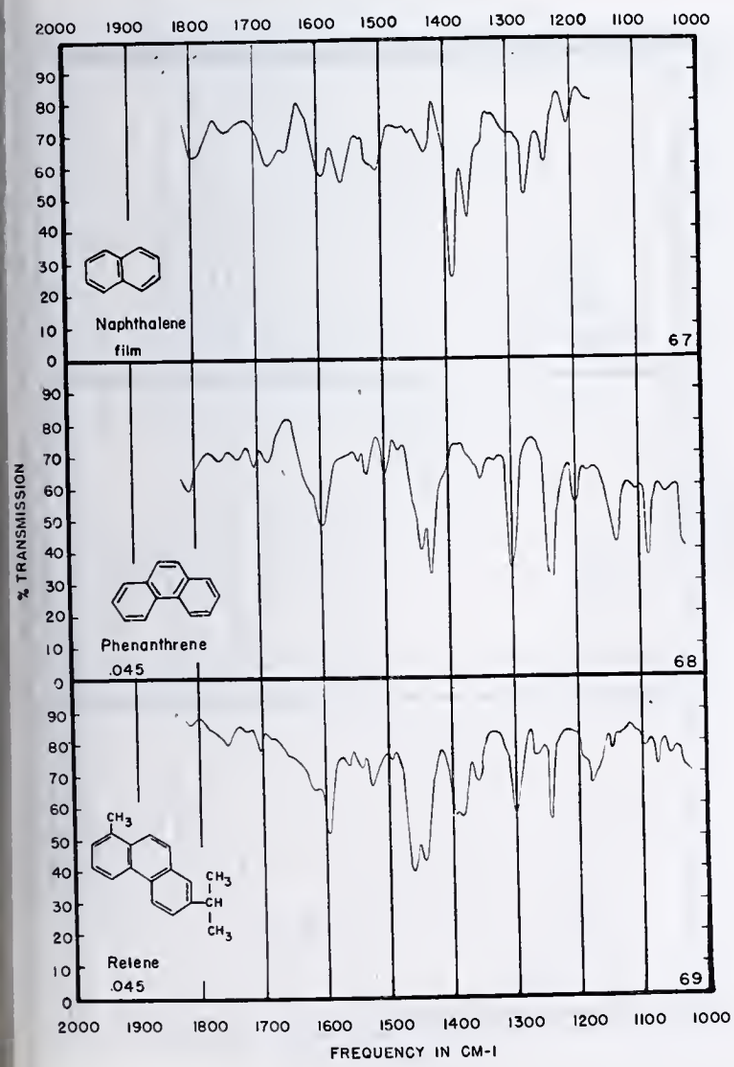

o-Xylene
.045
m-Xylene
.045
p-Xylene
.045
% TRANSMISSION
FREQUENCY IN CM-1

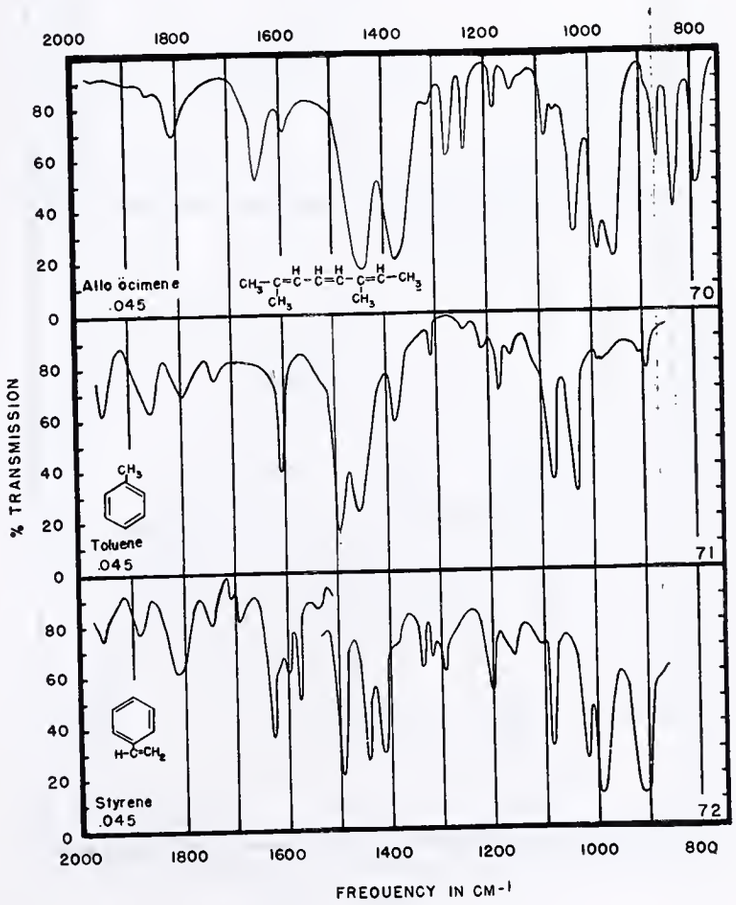

Benzene & Xylol
.150
55
1,2,3-Trimethyl benzene
.02
56
1,2,4-Trimethyl benzene
.02
57
% TRANSMISSION
FREQUENCY IN CM-1

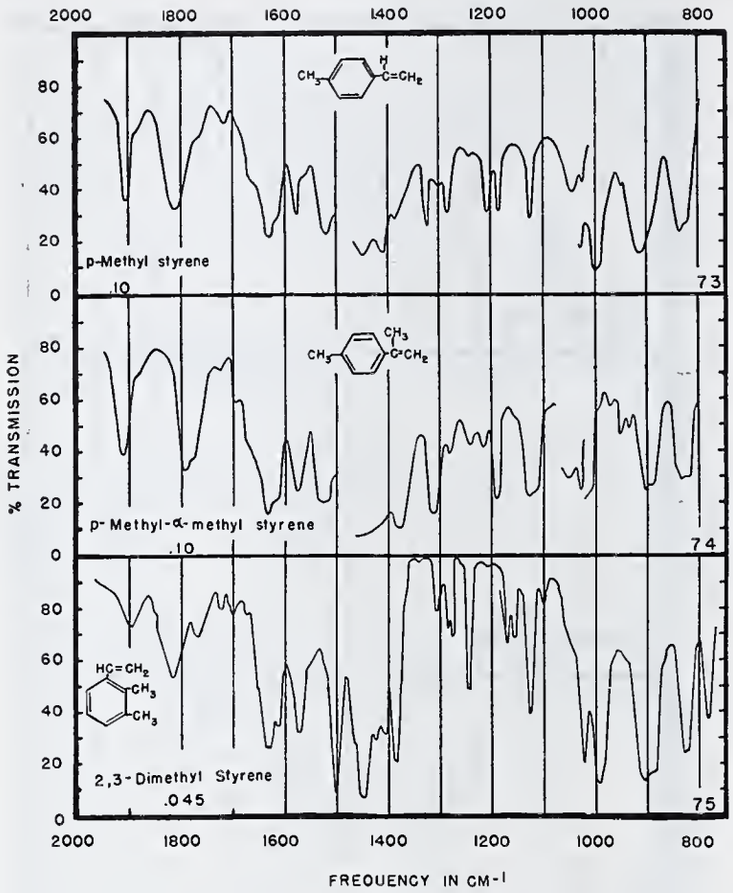

1,3,5-Trimethyl benzene
.02
58
Diphenyl
.028
59
Dibenzyl
.045
60
% TRANSMISSION
FREQUENCY IN CM-1

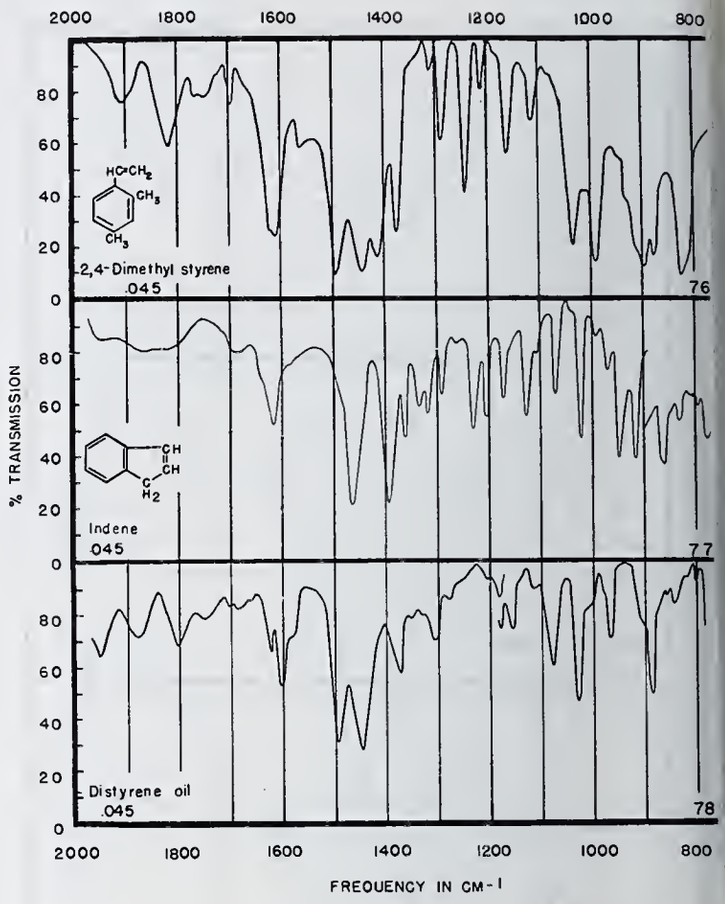

Stilbene cap
61
Triphenyl methane .005
62
α-Diphenyl benzene .005
63
% TRANSMISSION
FREQUENCY IN CM-1

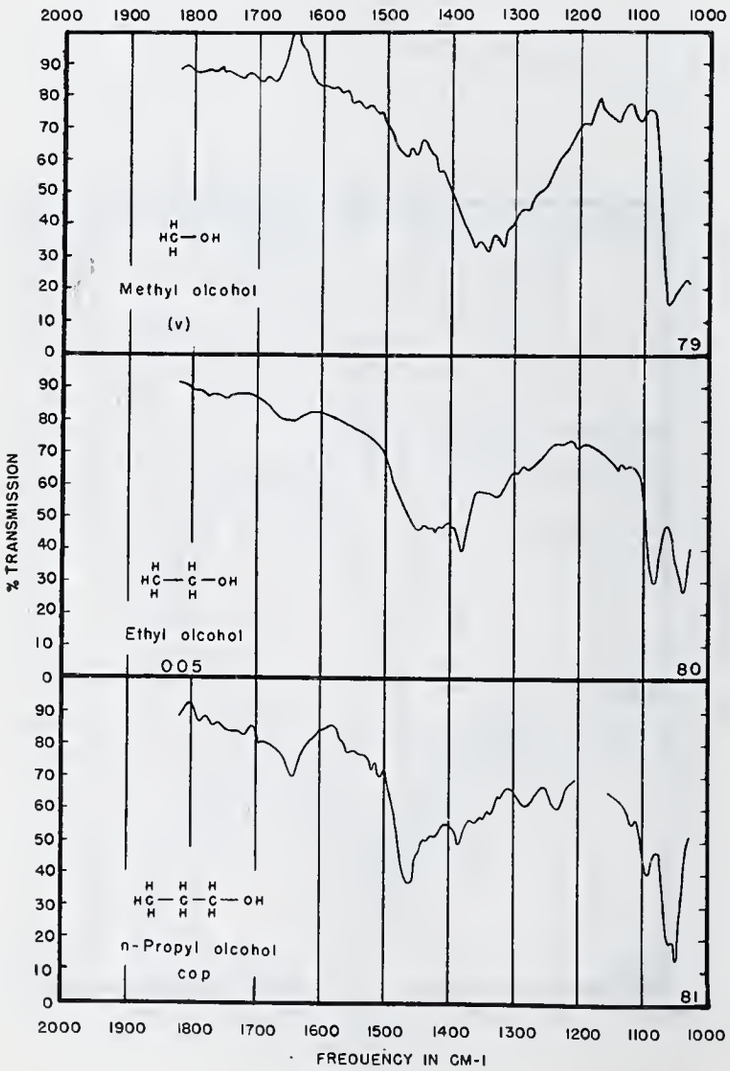

m-Diphenyl benzene .035
64
p-Diphenyl benzene .035
65
1,3,5-Triphenyl benzene .005
66
% TRANSMISSION
FREQUENCY IN CM-1

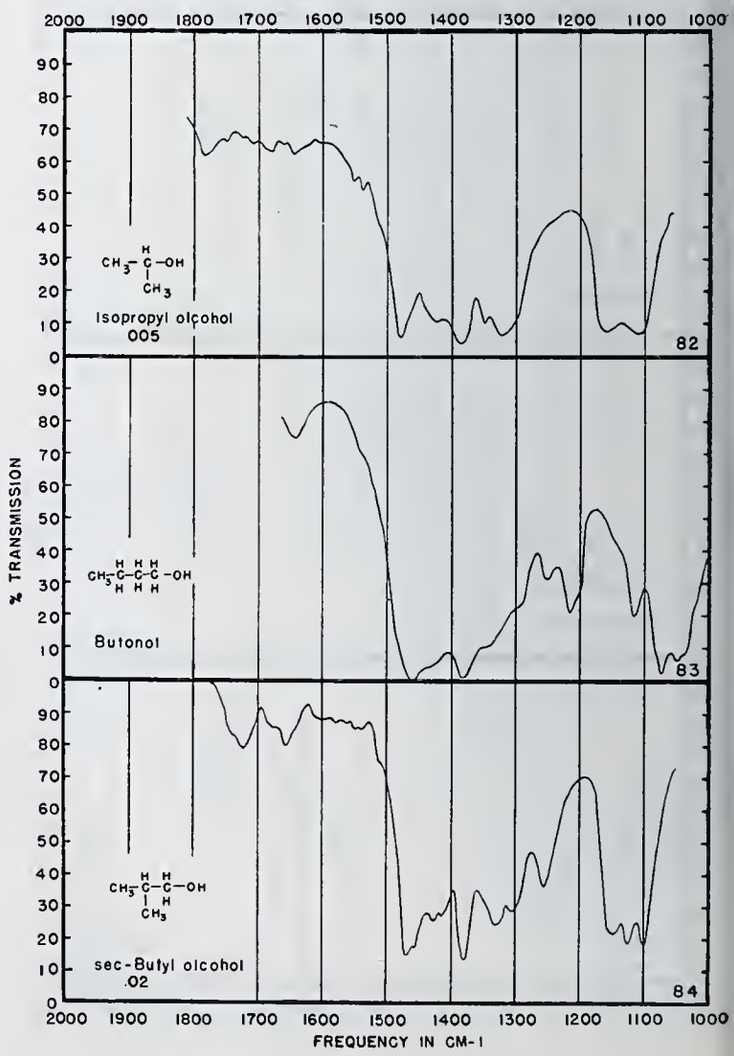

Naphthalene film
67
Phenanthrene .045
68
Retene .045
69
% TRANSMISSION
FREQUENCY IN CM-1

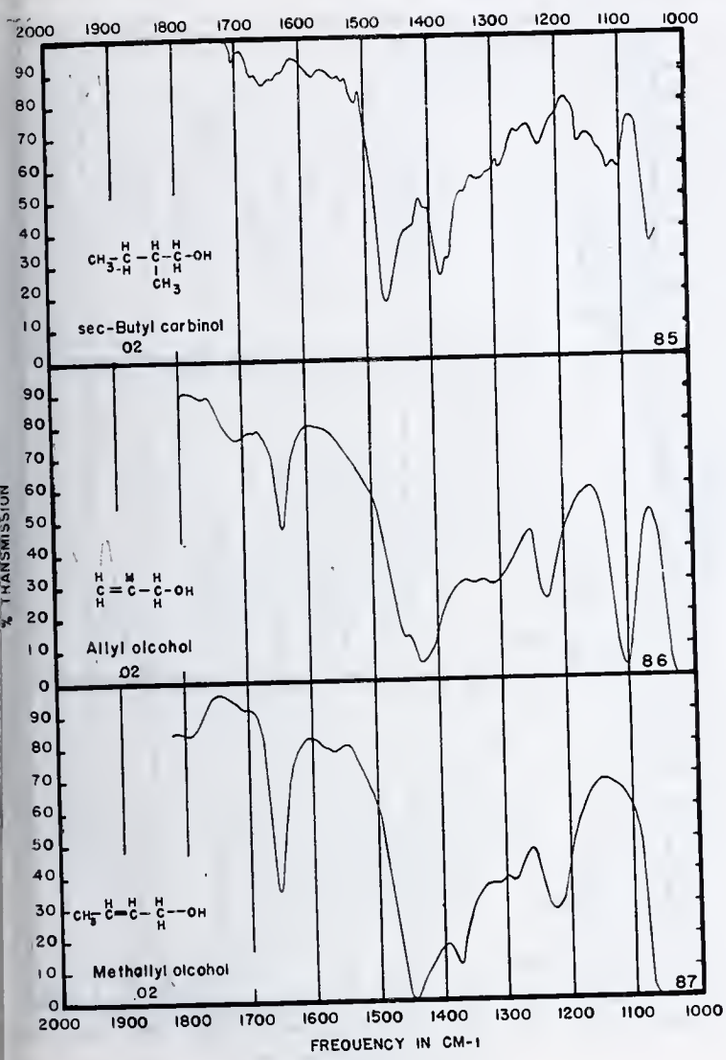

Allo ocimene .045
70
Toluene .045
71
Styrene .045
72
% TRANSMISSION
FREQUENCY IN CM-1

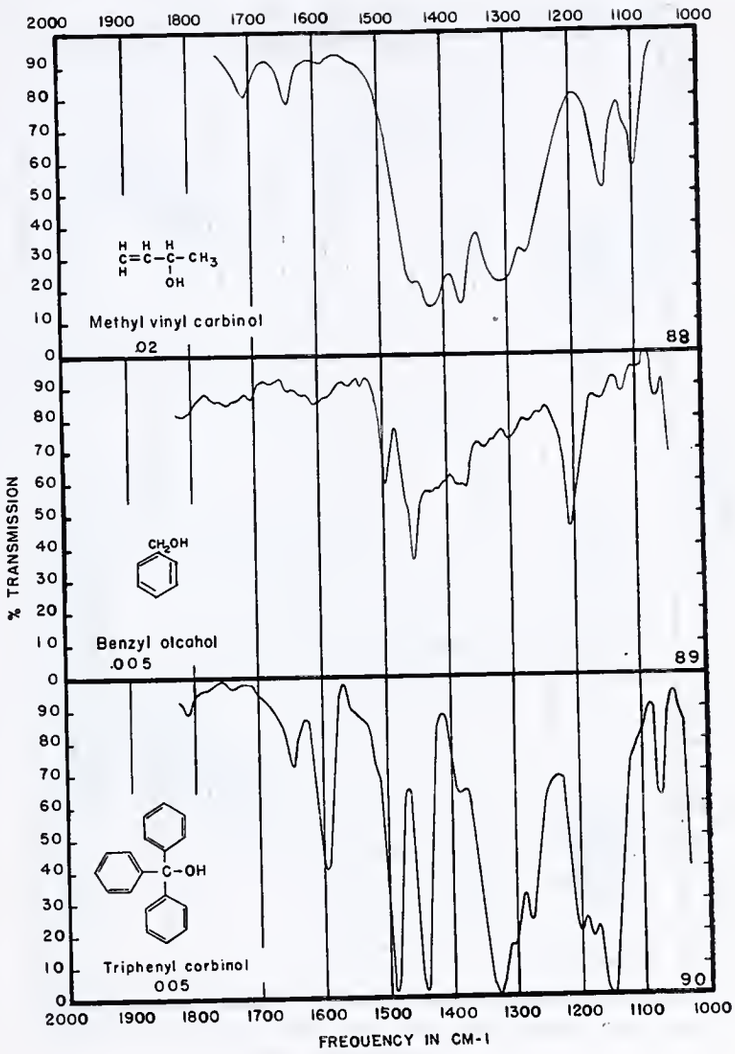

p-Methyl styrene
.10
73
p-Methyl-α-methyl styrene
.10
74
2,3-Dimethyl Styrene
.045
75
% TRANSMISSION
FREQUENCY IN CM-1

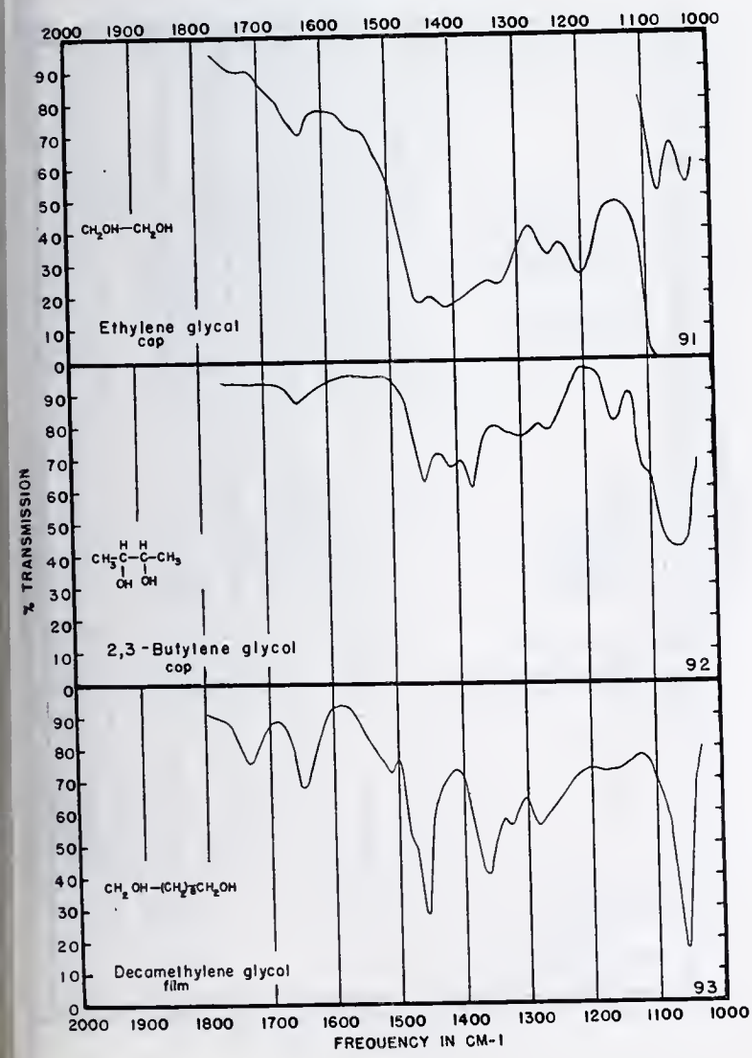

2,4-Dimethyl styrene
.045
76
Indene
.045
77
Distyrene oil
.045
78
% TRANSMISSION
FREQUENCY IN CM-1

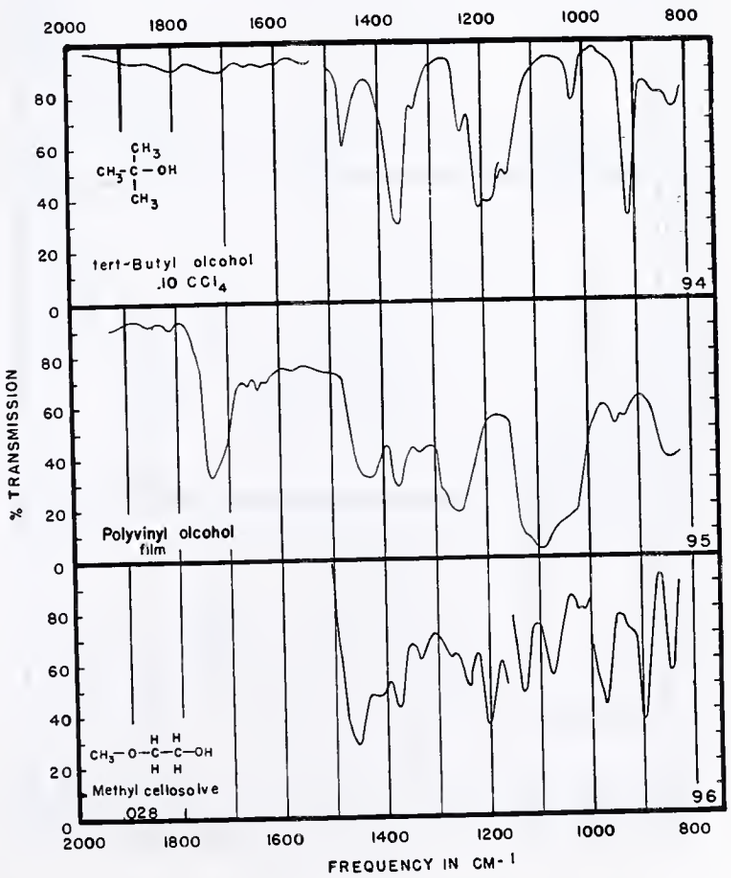

Methyl alcohol
(v)
79
Ethyl alcohol
.005
80
n-Propyl alcohol
.cop
81
% TRANSMISSION
FREQUENCY IN CM-1

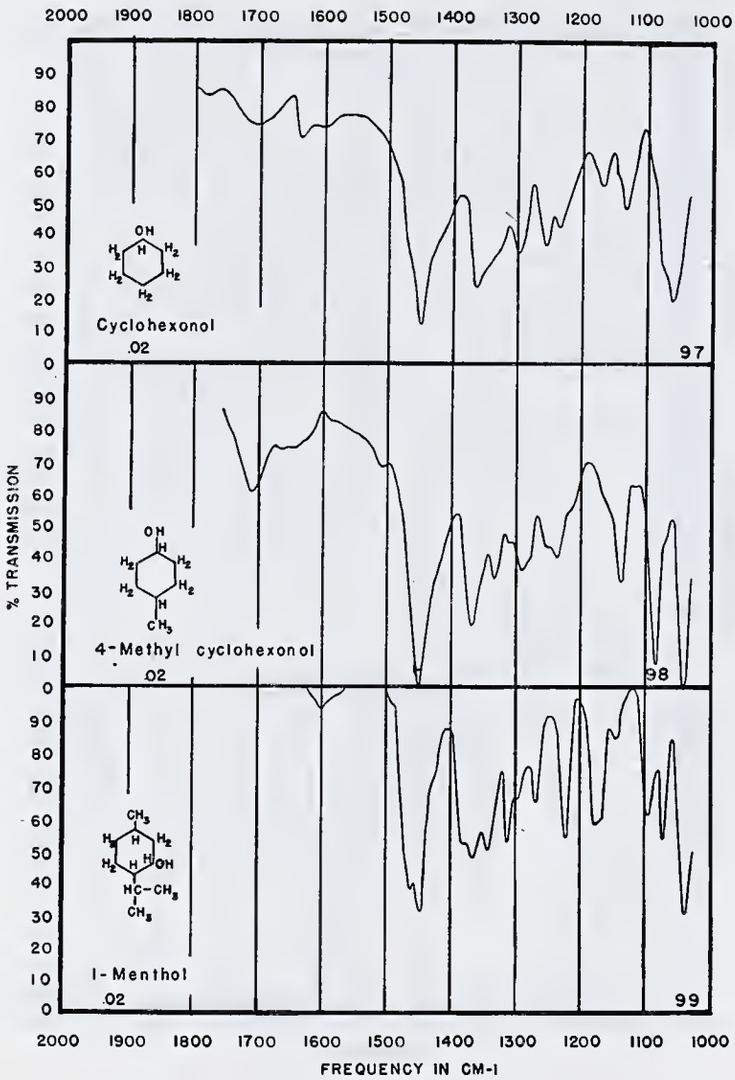

Isopropyl alcohol
.005
82
Butanol
83
sec-Butyl alcohol
.02
84
% TRANSMISSION
FREQUENCY IN CM-1

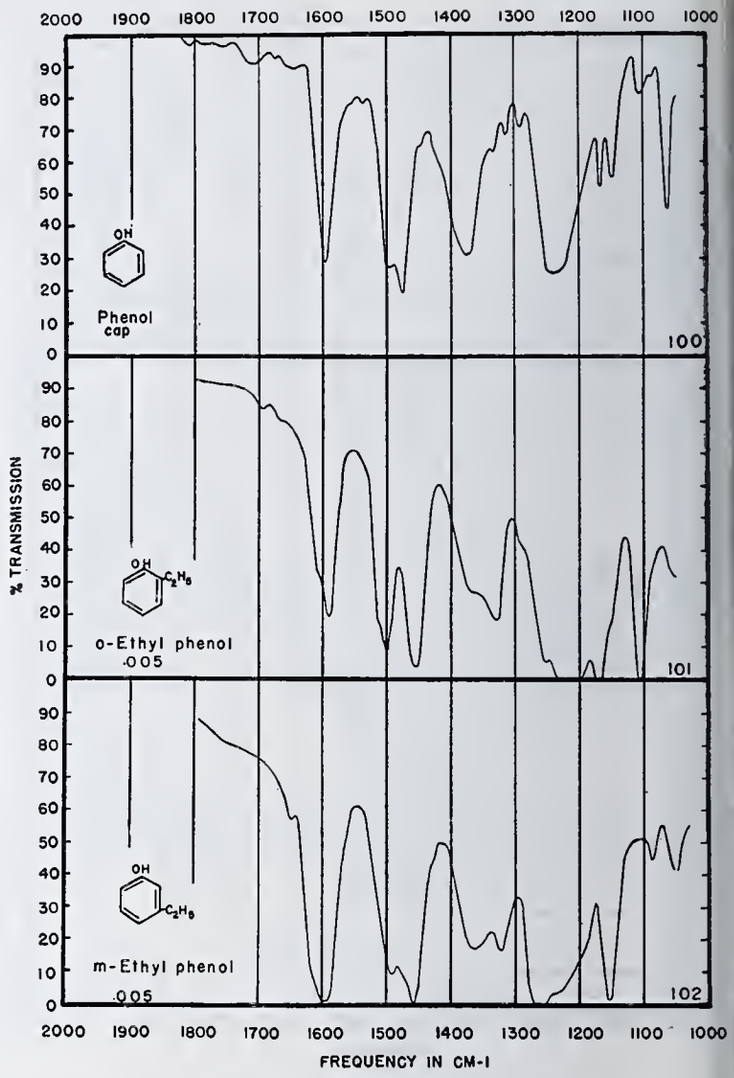

sec-Butyl carbinol
.02
85
Allyl alcohol
.02
86
Methallyl alcohol
.02
87
% TRANSMISSION
FREQUENCY IN CM-1

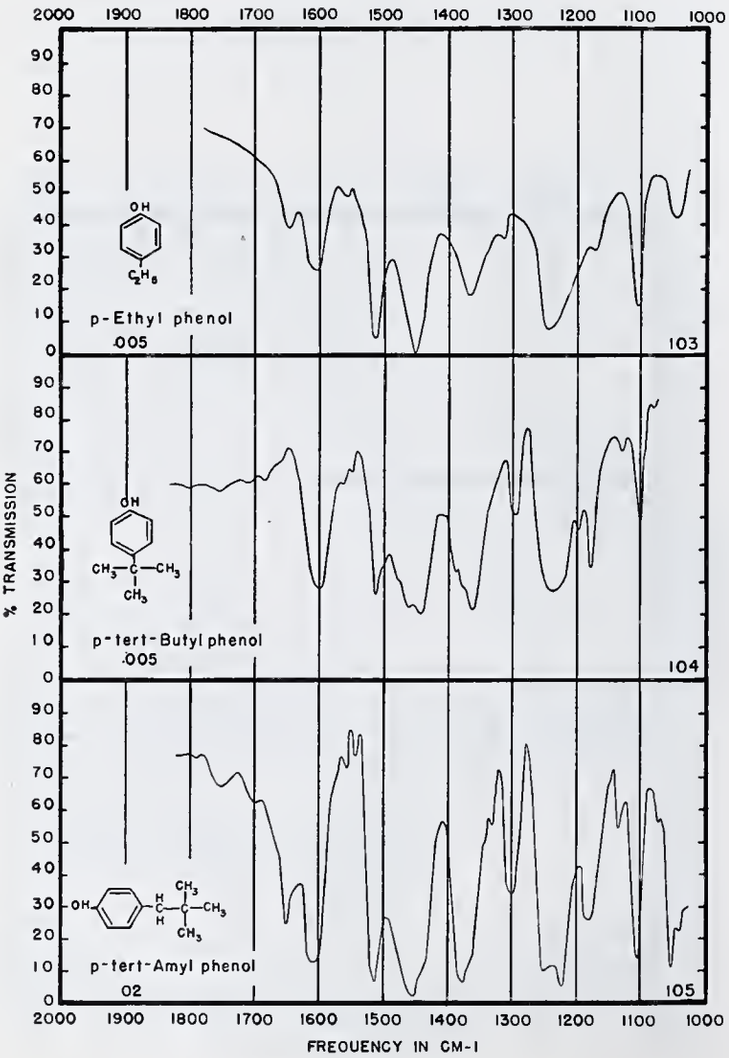

Methyl vinyl carbinol
.02
88
Benzyl alcohol
.005
89
Triphenyl carbinol
.005
90
% TRANSMISSION
FREQUENCY IN CM-1

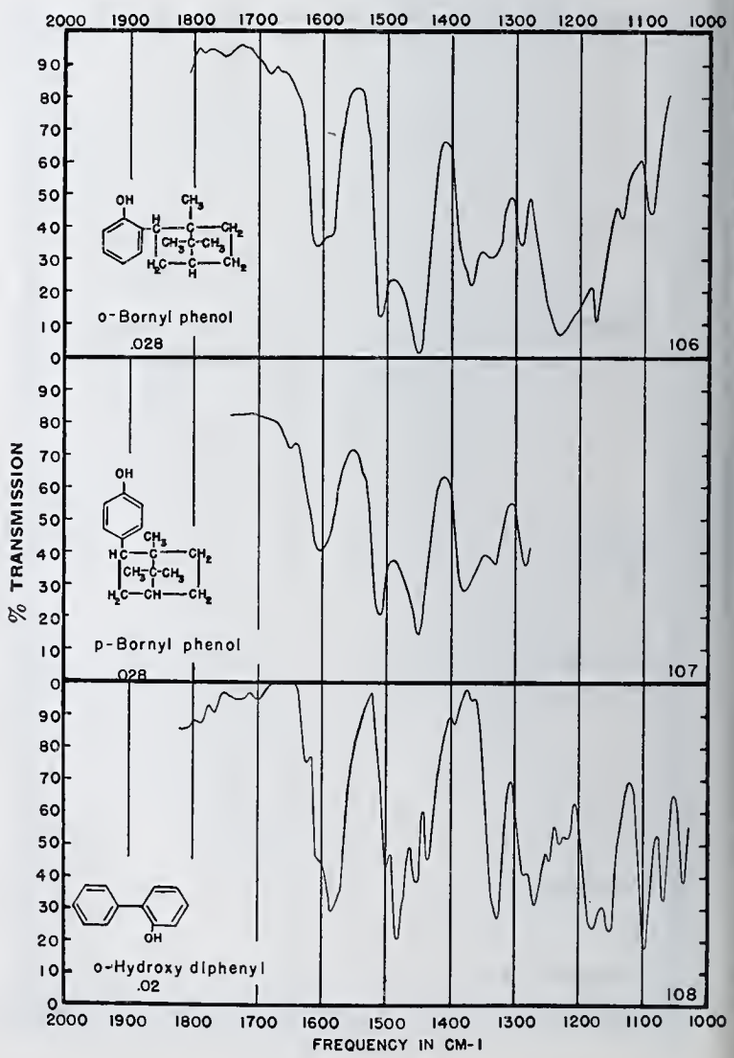

Ethylene glycol
cap
91
2,3-Butylene glycol
cap
92
Decamethylene glycol
film
93
% TRANSMISSION
FREQUENCY IN CM-1

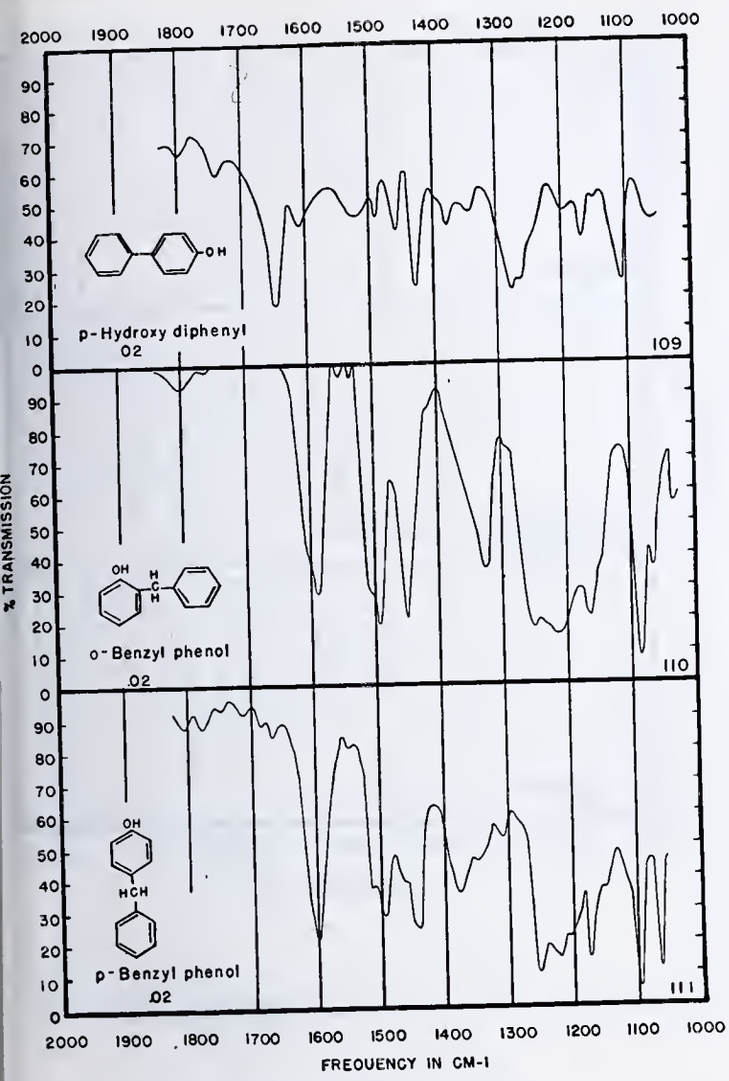

tert-Butyl alcohol
.10 CCl4
94
Polyvinyl alcohol
film
95
Methyl cellosolve
.028
96
% TRANSMISSION
FREQUENCY IN CM-1

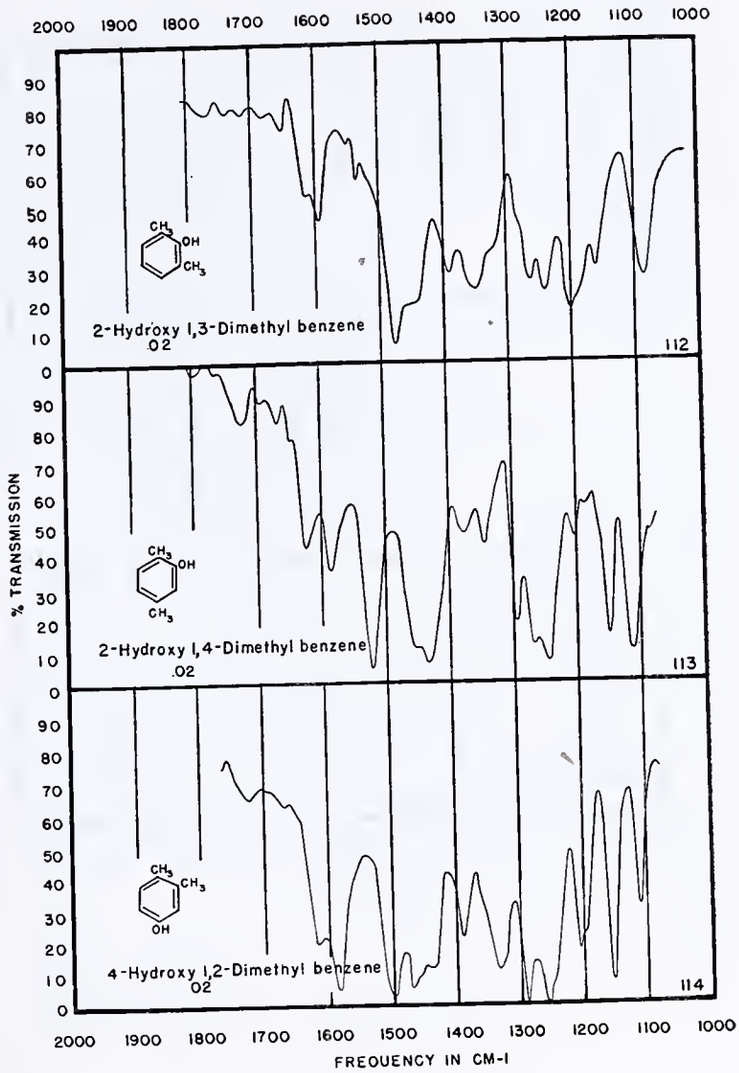

Cyclohexonol
.02
97
4-Methyl cyclohexonol
.02
98
l-Menthol
.02
99
% TRANSMISSION
FREQUENCY IN CM-1

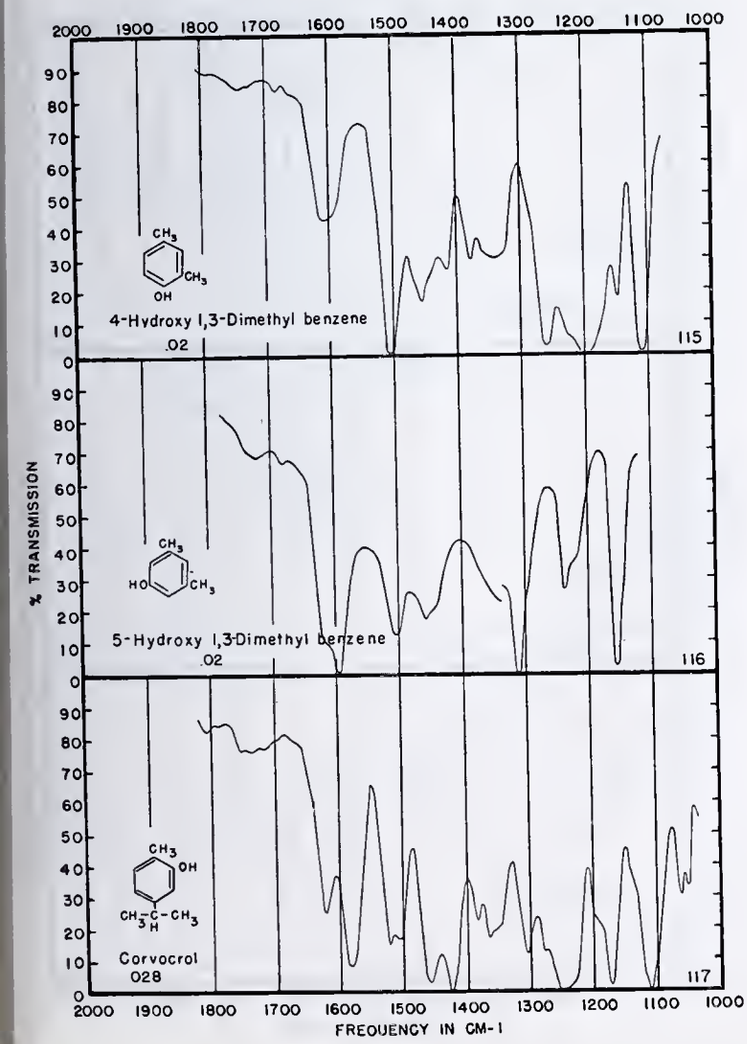

Phenol
cap
100
o-Ethyl phenol
.005
101
m-Ethyl phenol
.005
102
% TRANSMISSION
FREQUENCY IN CM-1

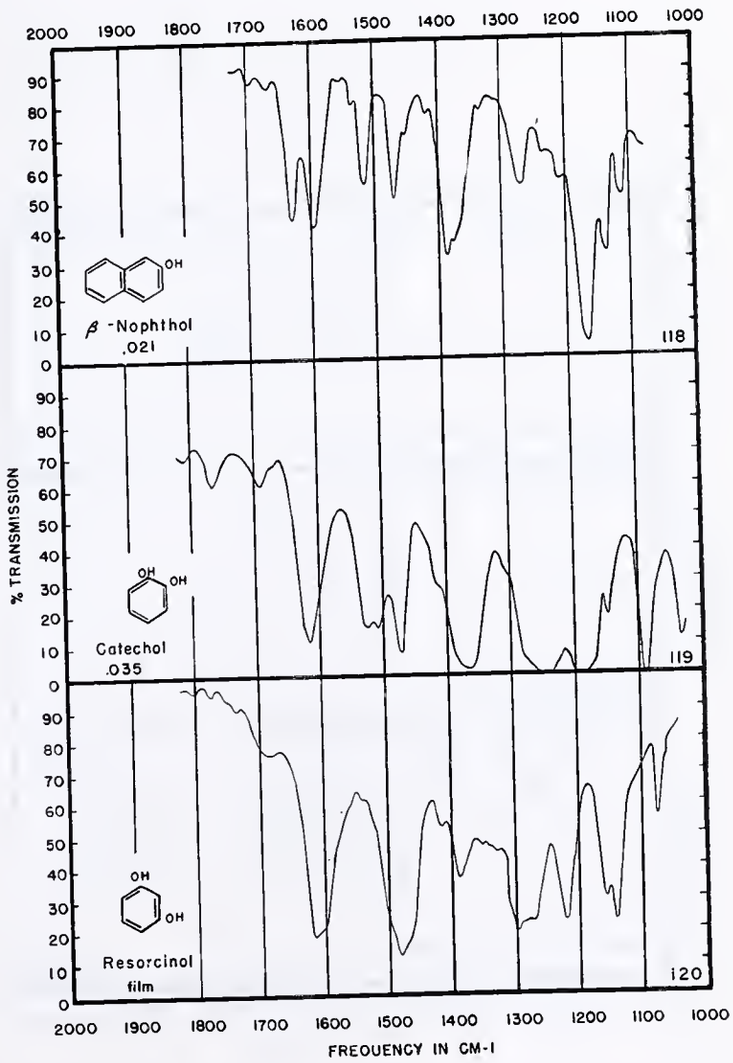

p-Ethyl phenol
.005
103
p-tert-Butyl phenol
.005
104
p-tert-Amyl phenol
.02
105
% TRANSMISSION
FREQUENCY IN CM-1

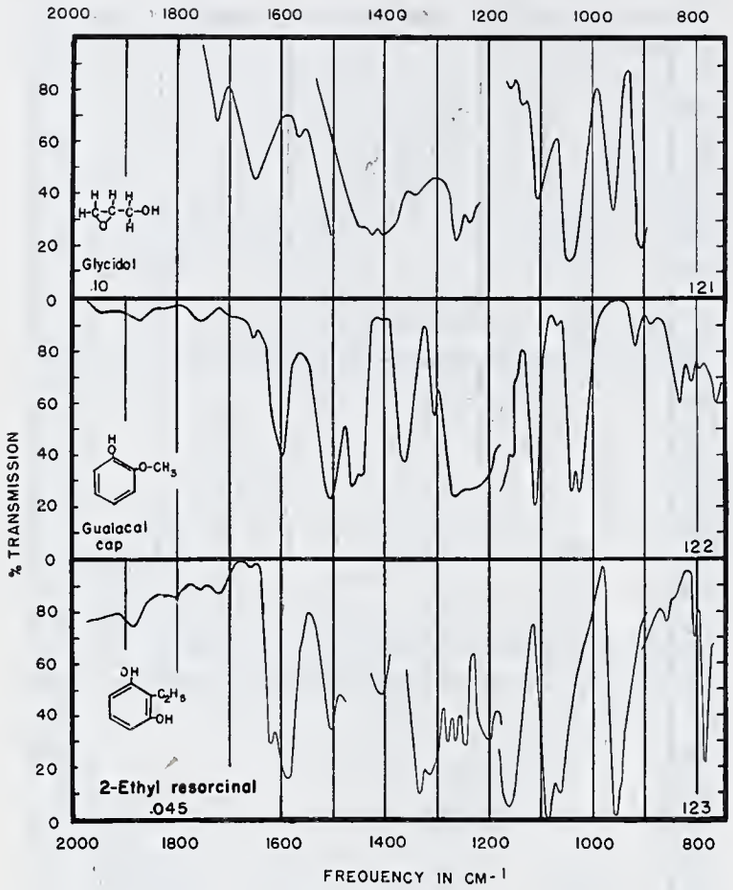

o-Bornyl phenol
.028
106
p-Bornyl phenol
.028
107
o-Hydroxy diphenyl
.02
108
% TRANSMISSION
FREQUENCY IN CM-1

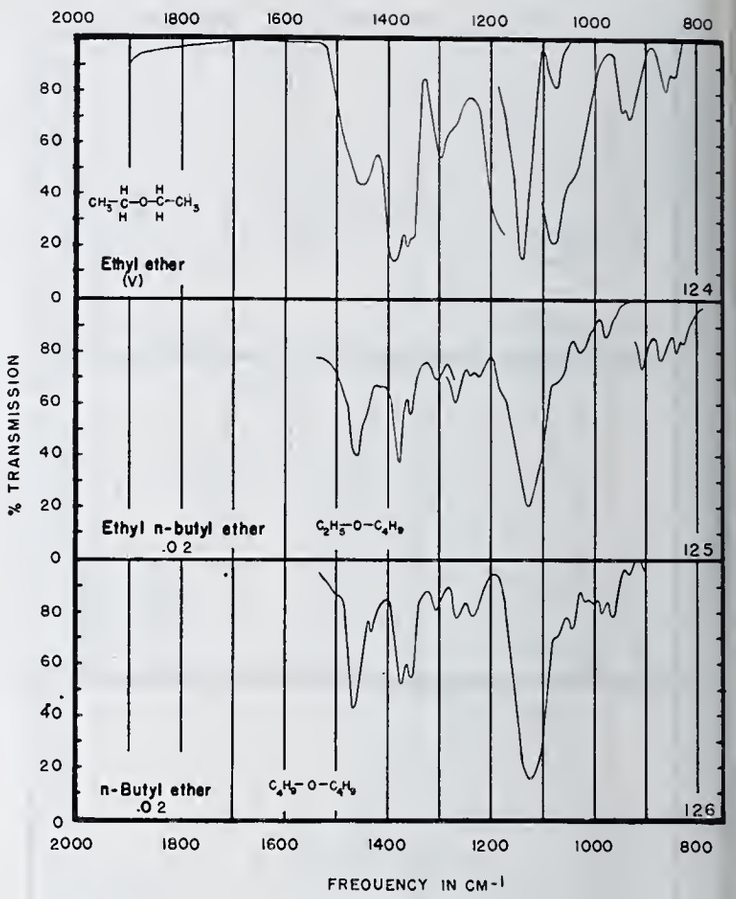

p-Hydroxy diphenyl
.02
109
o-Benzyl phenol
.02
110
p-Benzyl phenol
.02
111
% TRANSMISSION
FREQUENCY IN CM-1

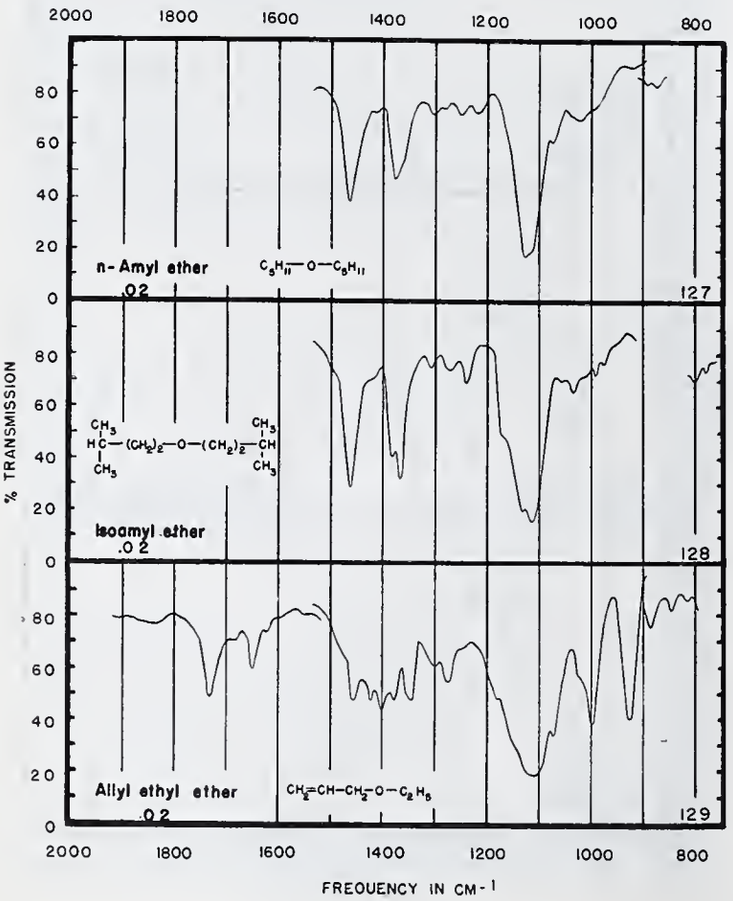

2-Hydroxy 1,3-Dimethyl benzene
.02
112
2-Hydroxy 1,4-Dimethyl benzene
.02
113
4-Hydroxy 1,2-Dimethyl benzene
.02
114
% TRANSMISSION
FREQUENCY IN CM-1

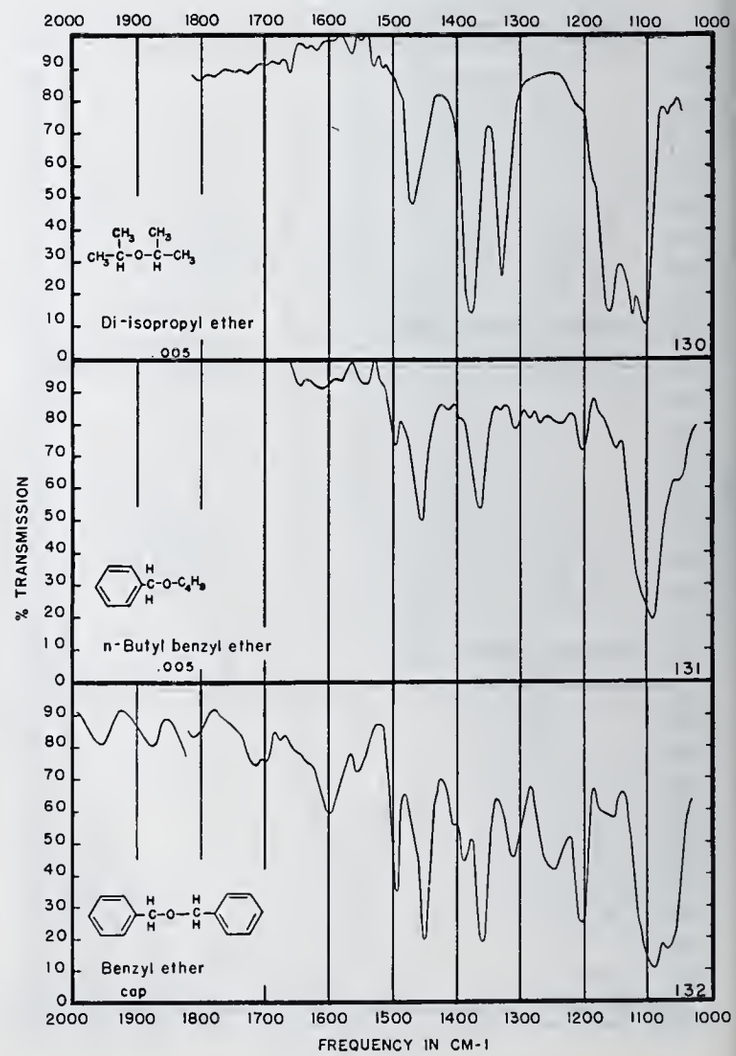

4-Hydroxy 1,3-Dimethyl benzene
.02
115
5-Hydroxy 1,3-Dimethyl benzene
.02
116
Corvocrol
.028
117
% TRANSMISSION
FREQUENCY IN CM-1

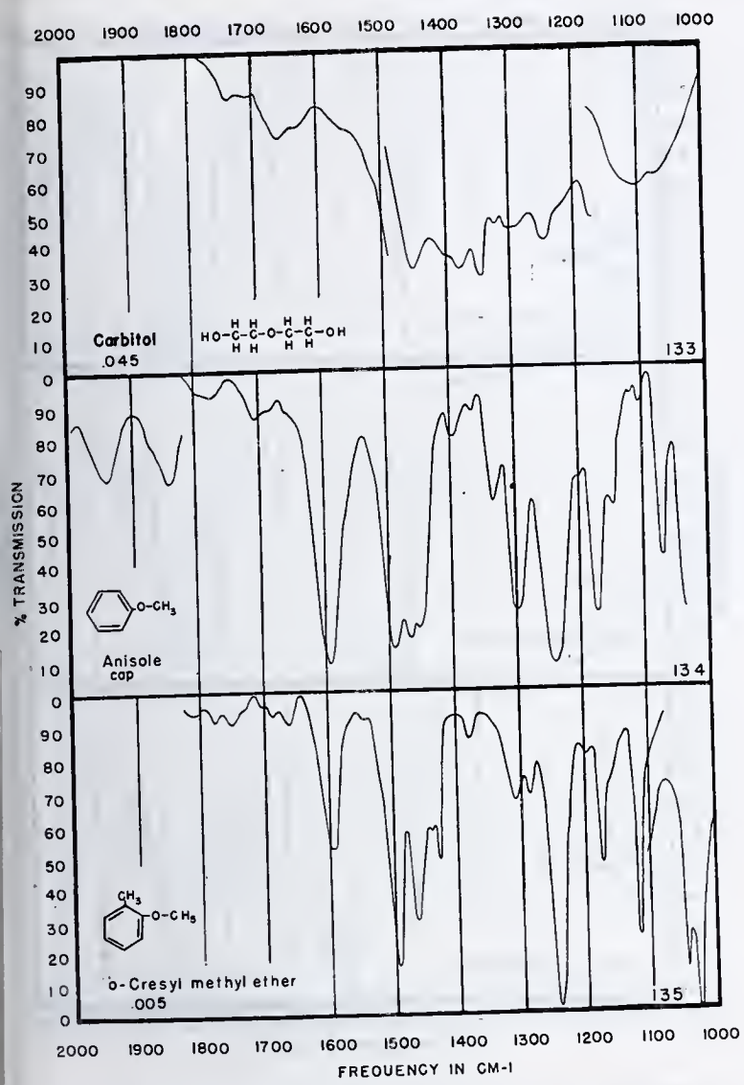

β-Nophthol
.021
118
Catechol
.035
119
Resorcinol
film
120
% TRANSMISSION
FREQUENCY IN CM-1

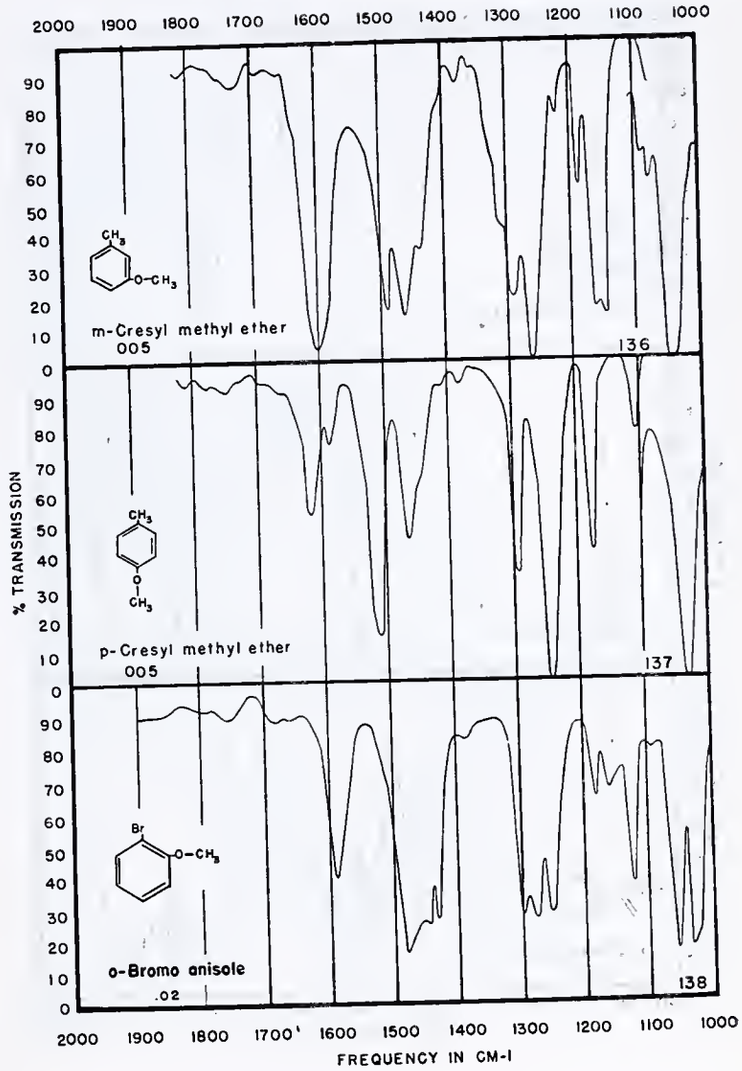

Glycidol
.10
121
Guaiacol
cap
122
2-Ethyl resorcinol
.045
123
% TRANSMISSION
FREQUENCY IN CM-1

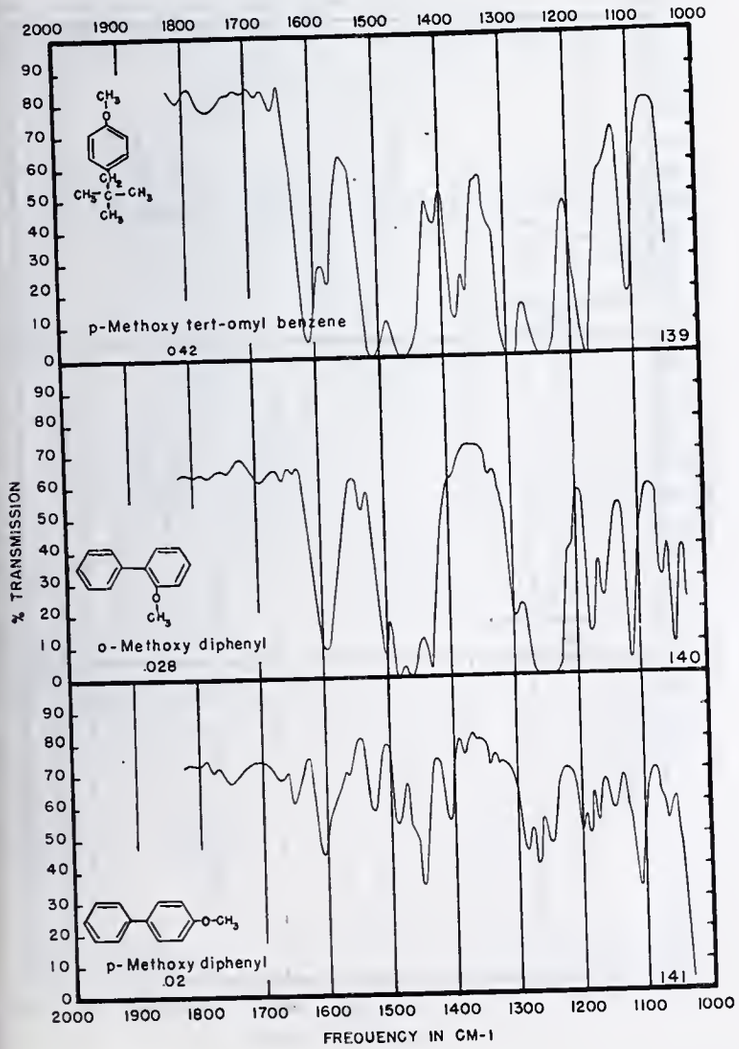

Ethyl ether
(V)
124
Ethyl n-butyl ether
.02
125
n-Butyl ether
.02
126
% TRANSMISSION
FREQUENCY IN CM-1

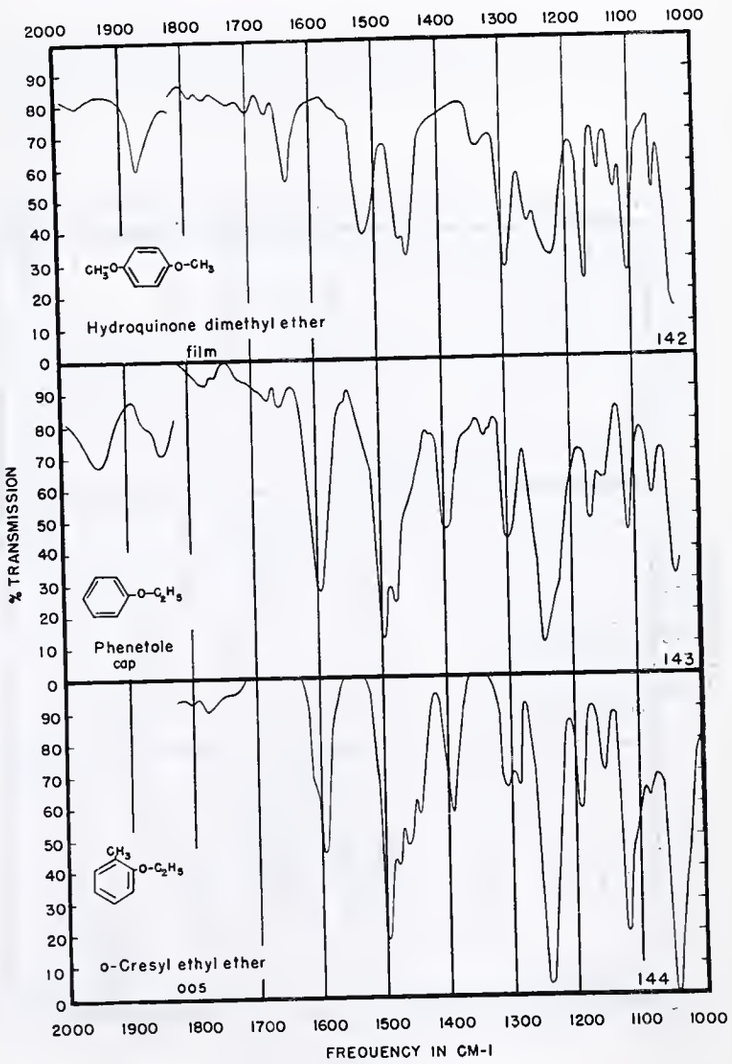

n-Amyl ether
.02
127
Isoamyl ether
.02
128
Allyl ethyl ether
.02
129
% TRANSMISSION
FREQUENCY IN CM-1

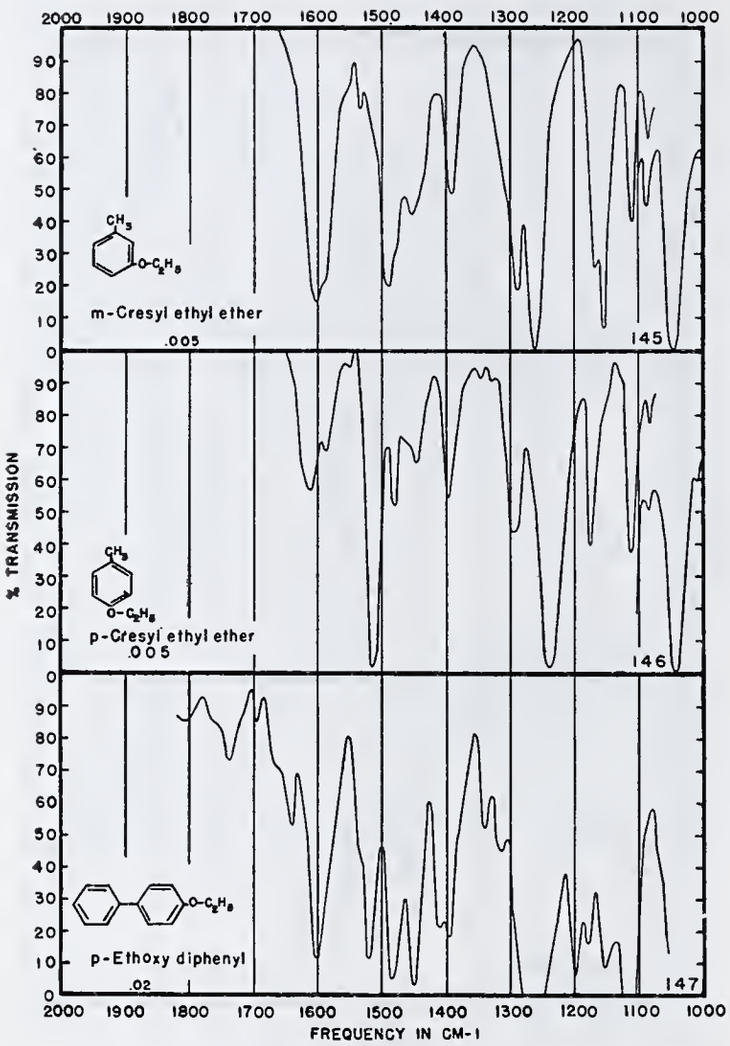

Di-isopropyl ether
.005
130
n-Butyl benzyl ether
.005
131
Benzyl ether
cap
132
% TRANSMISSION
FREQUENCY IN CM-1

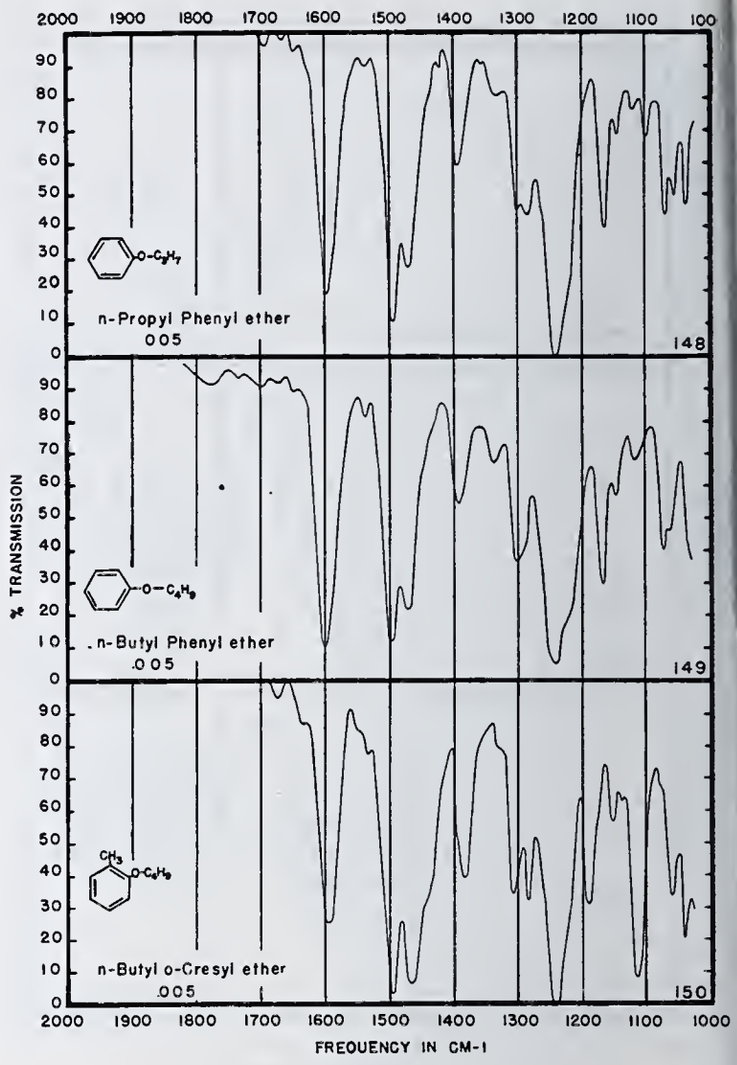

Carbitol
.045
133
Anisole
cap
134
o-Cresyl methyl ether
.005
135
% TRANSMISSION
FREQUENCY IN CM-1

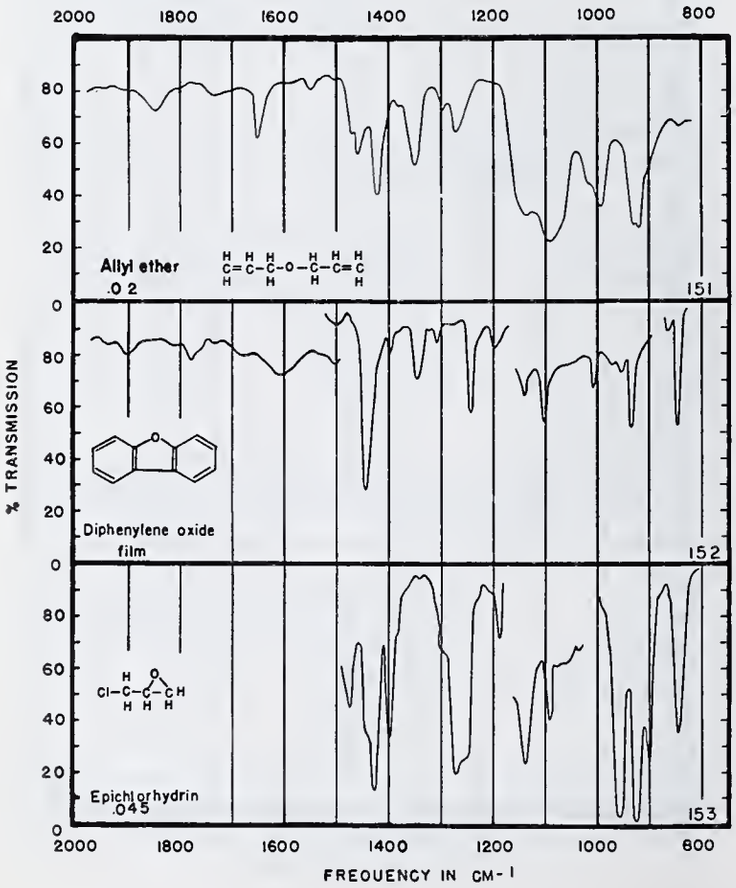

m-Cresyl methyl ether
005
136
p-Cresyl methyl ether
005
137
o-Bromo anisole
.02
138
% TRANSMISSION
FREQUENCY IN CM-1

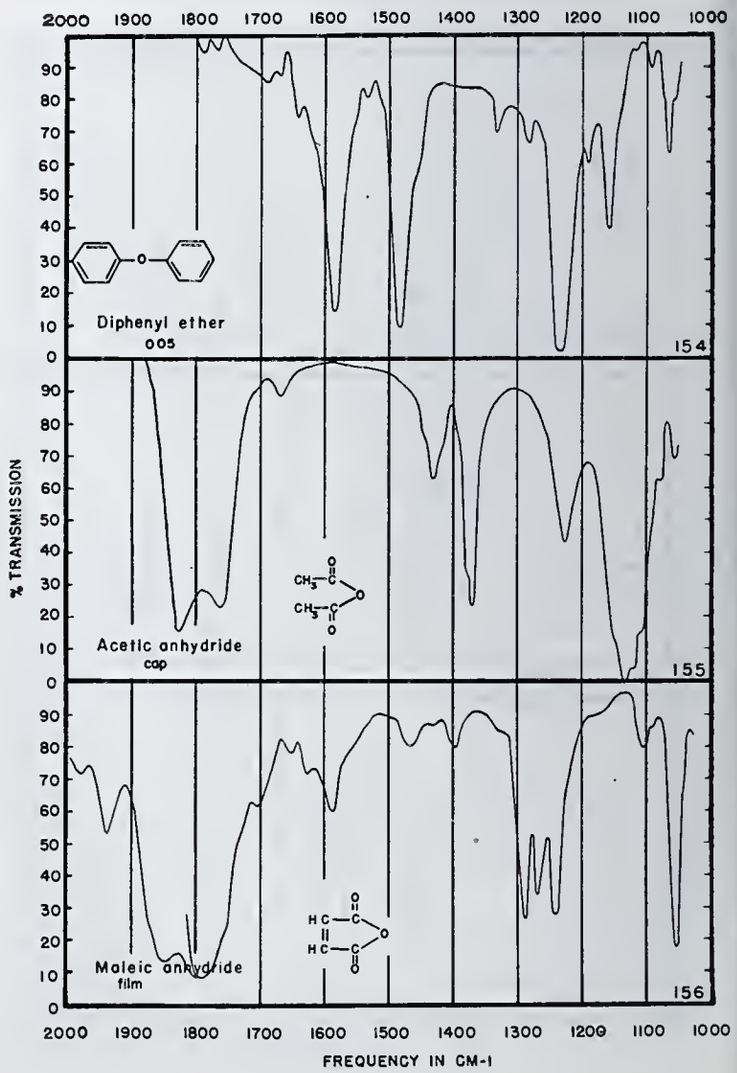

p-Methoxy tert-amyl benzene
042
139
o-Methoxy diphenyl
.028
140
p-Methoxy diphenyl
.02
141
% TRANSMISSION
FREQUENCY IN CM-1

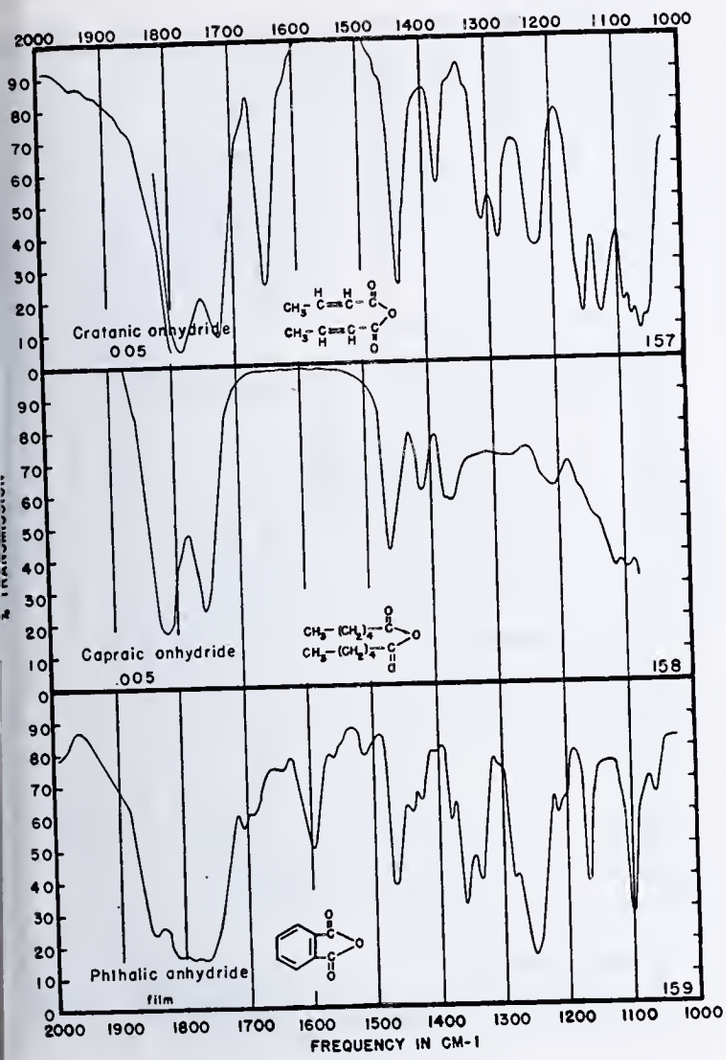

Hydroquinone dimethyl ether
film
142
Phenetole
cap
143
o-Cresyl ethyl ether
005
144
% TRANSMISSION
FREQUENCY IN CM-1

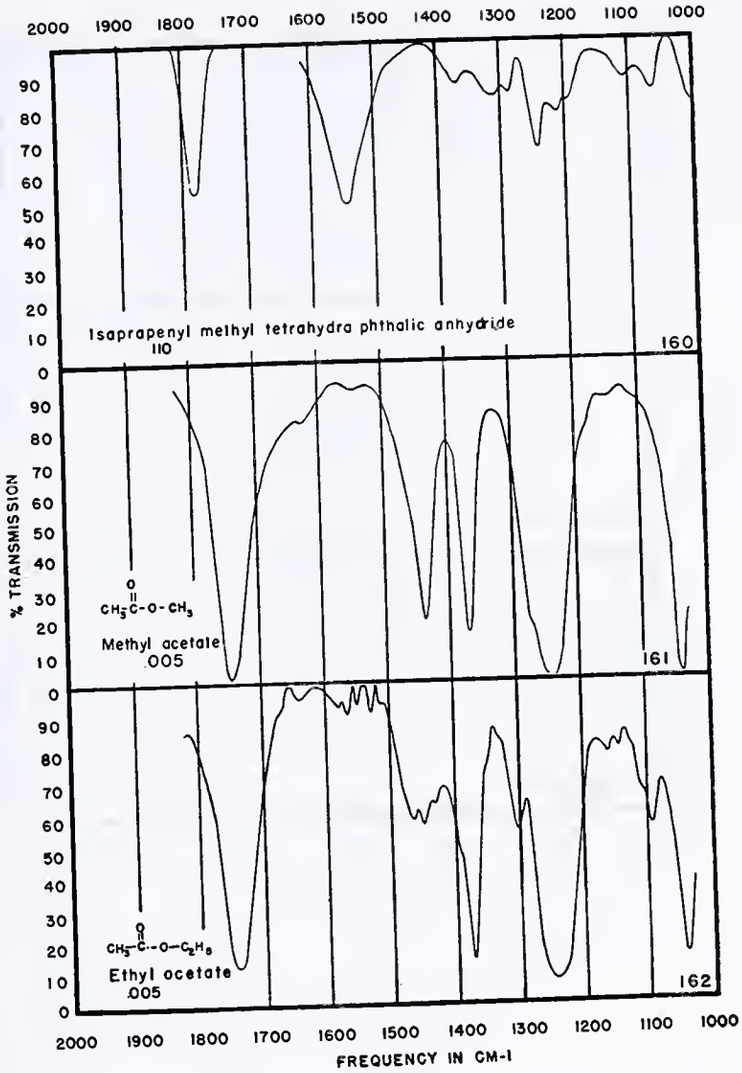

% TRANSMISSION
FREQUENCY IN CM-1
m-Cresyl ethyl ether
.005
145
p-Cresyl ethyl ether
.005
146
p-Ethoxy diphenyl
.02
147

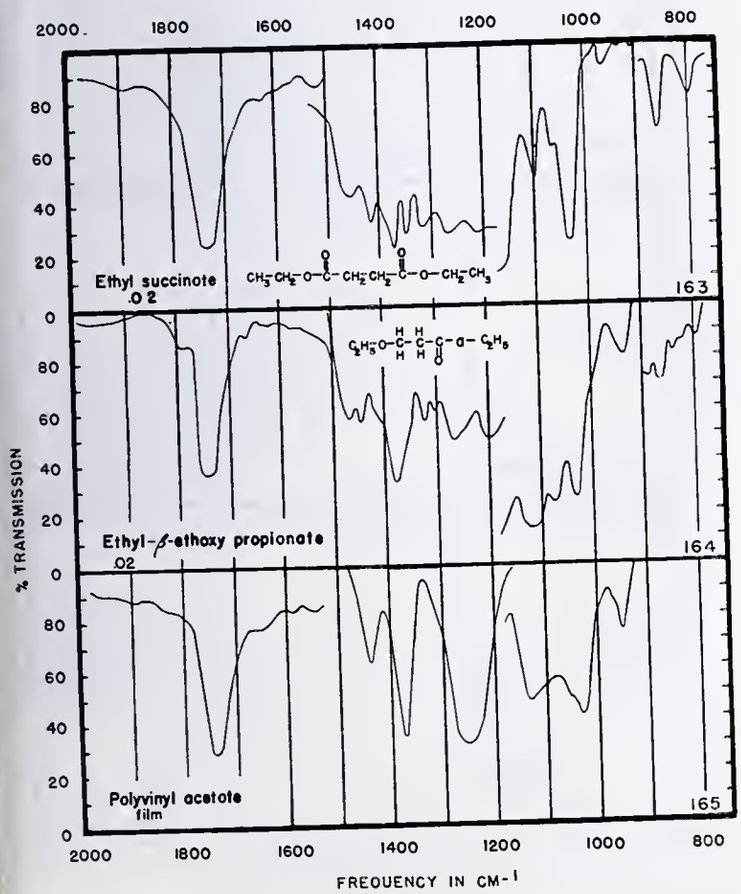

% TRANSMISSION
FREQUENCY IN CM-1
n-Propyl Phenyl ether
005
148
n-Butyl Phenyl ether
.005
149
n-Butyl o-Cresyl ether
.005
150

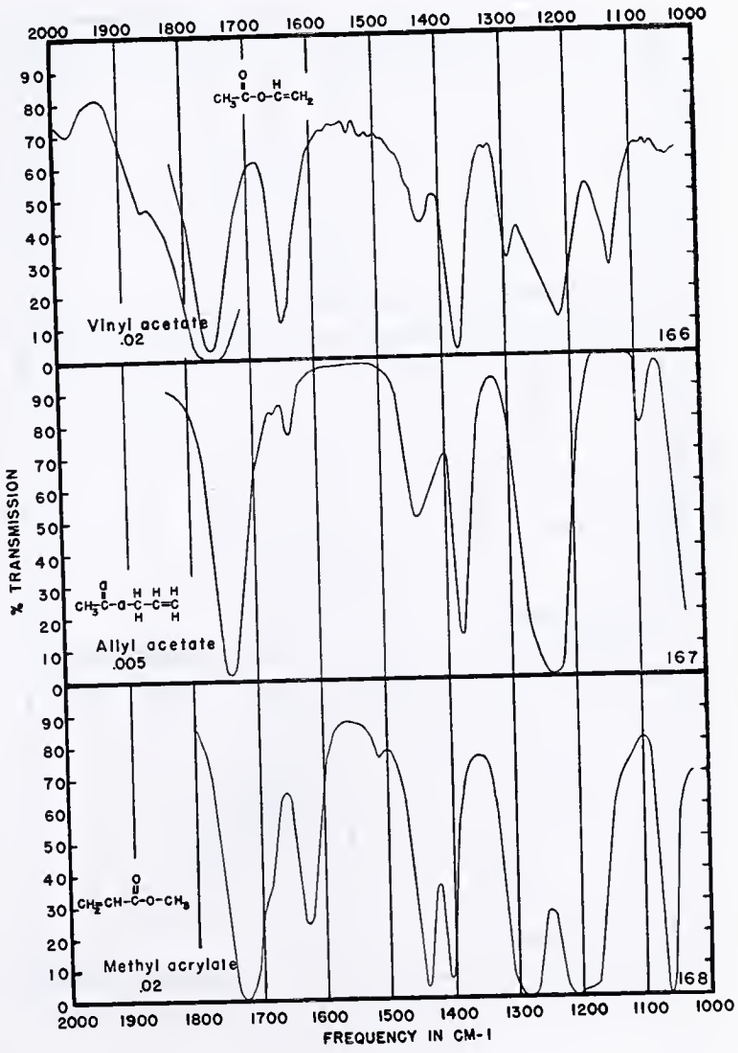

% TRANSMISSION
FREQUENCY IN CM-1
Allyl ether
.02
151
Diphenylene oxide
film
152
Epichlorhydrin
.045
153

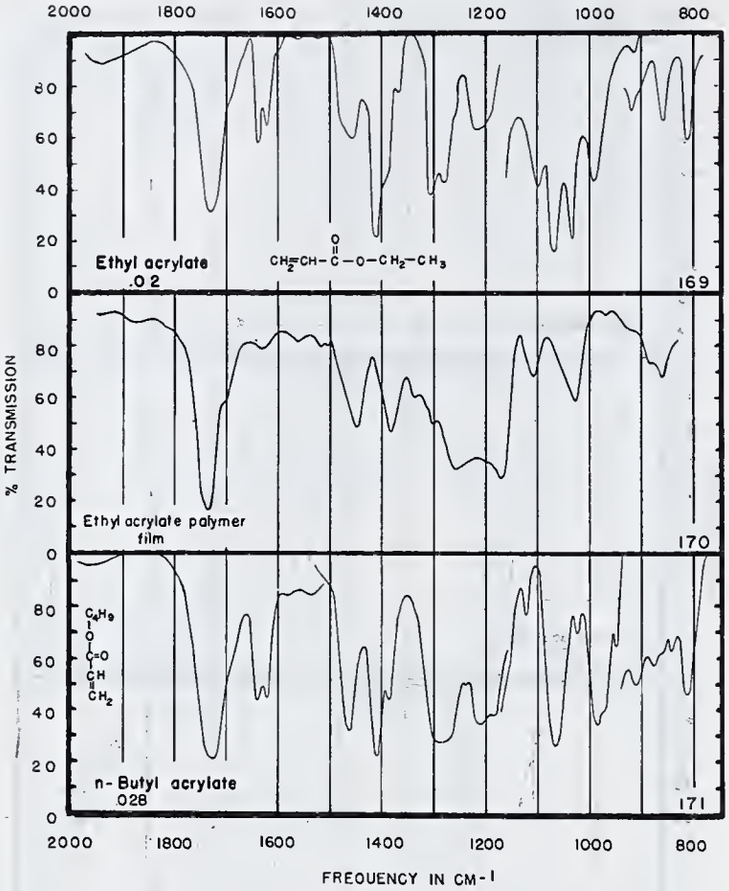

% TRANSMISSION
FREQUENCY IN CM-1
Diphenyl ether
005
154
Acetic anhydride
cap
155
Maleic anhydride
film
156

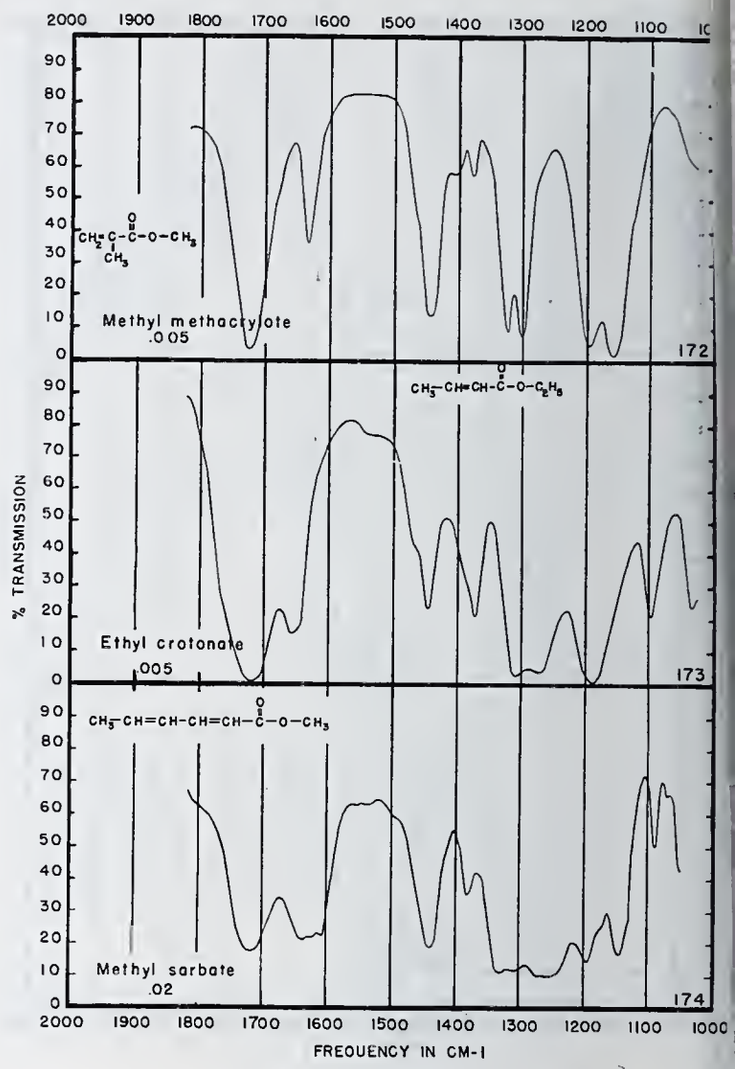

Cratanic anhydride
.005
157
Capraic anhydride
.005
158
Phthalic anhydride
film
159
FREQUENCY IN CM-1

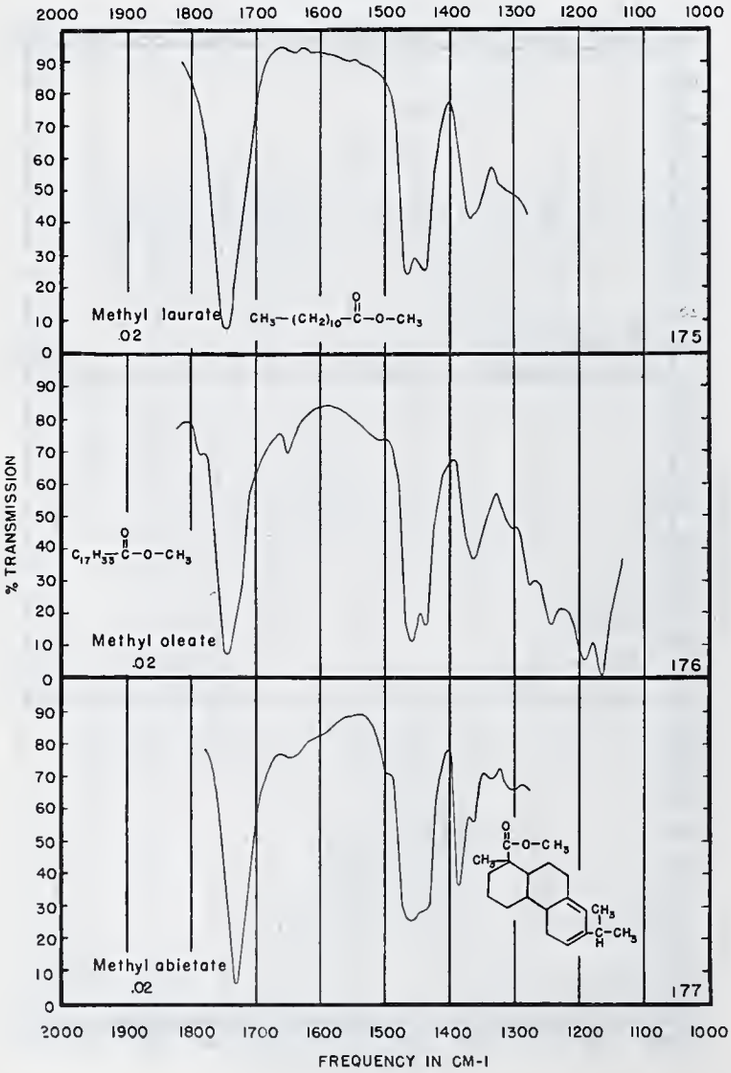

Isaprapenyl methyl tetrahydra phthalic anhydride
110
160
% TRANSMISSION
Methyl acetate
.005
161
Ethyl acetate
.005
162
FREQUENCY IN CM-1

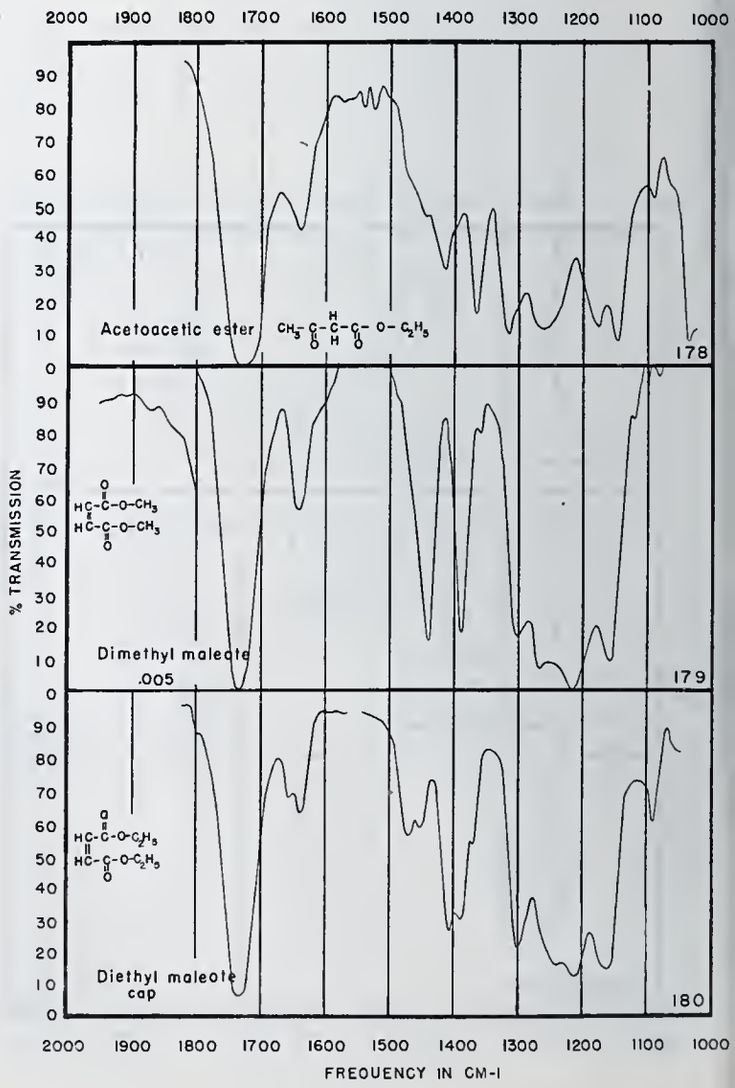

Ethyl succinate
.02
163
Ethyl-β-ethoxy propionate
.02
164
% TRANSMISSION
Polyvinyl acetate
film
165
FREQUENCY IN CM-1

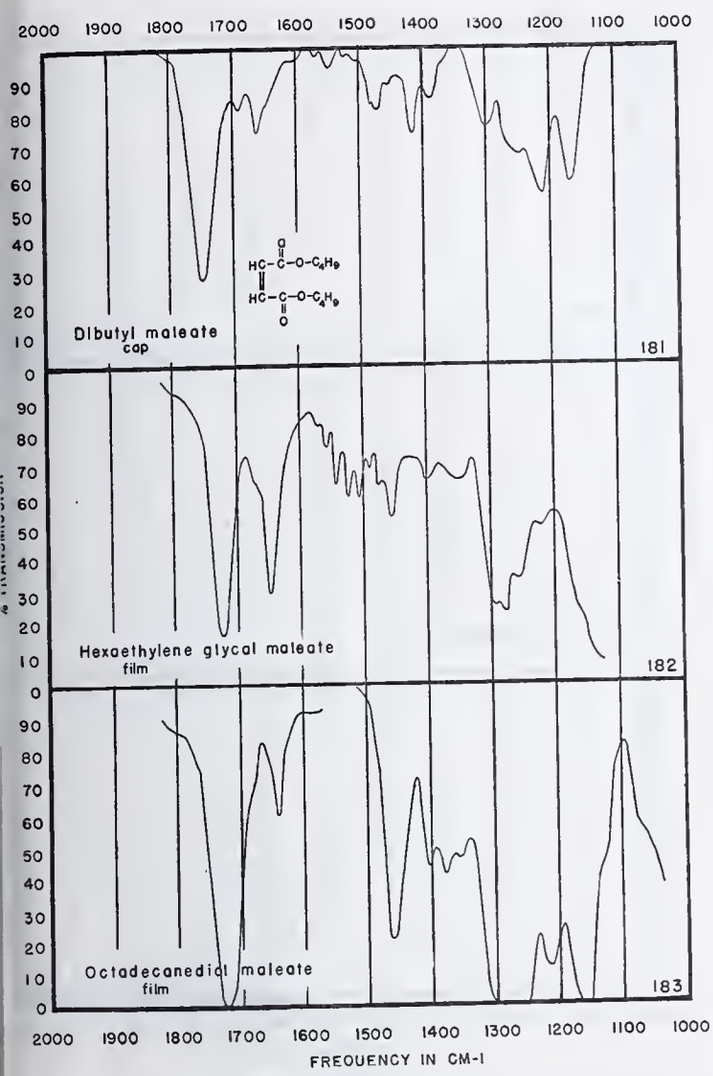

Vinyl acetate
.02
166
% TRANSMISSION
Allyl acetate
.005
167
Methyl acrylate
.02
168
FREQUENCY IN CM-1

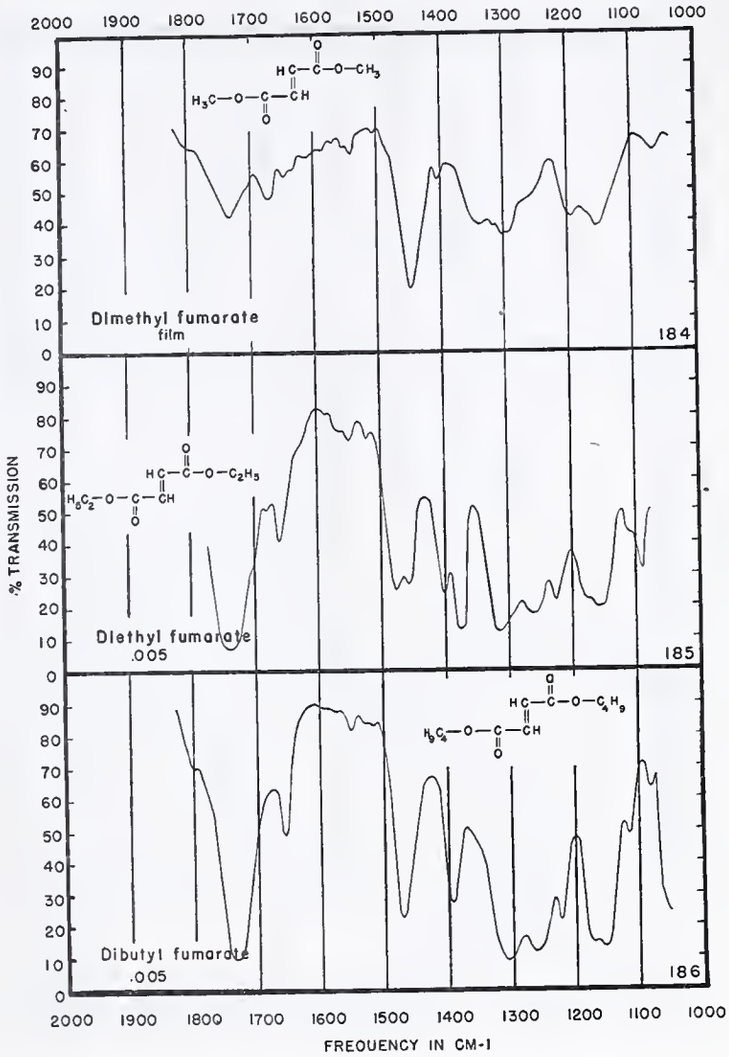

Ethyl acrylate
.02
169
Ethyl acrylate polymer
film
170
n-Butyl acrylate
.028
171
% TRANSMISSION
FREQUENCY IN CM-1

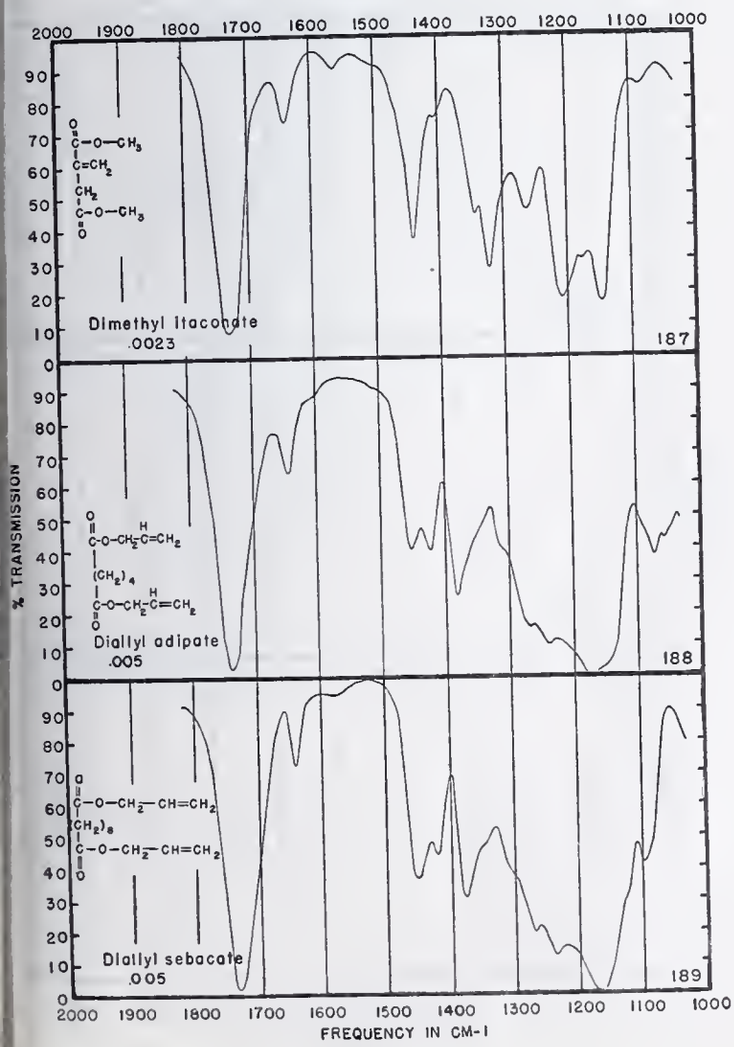

Methyl methacrylate
.005
172
Ethyl crotonate
.005
173
Methyl sarbate
.02
174
% TRANSMISSION
FREQUENCY IN CM-1

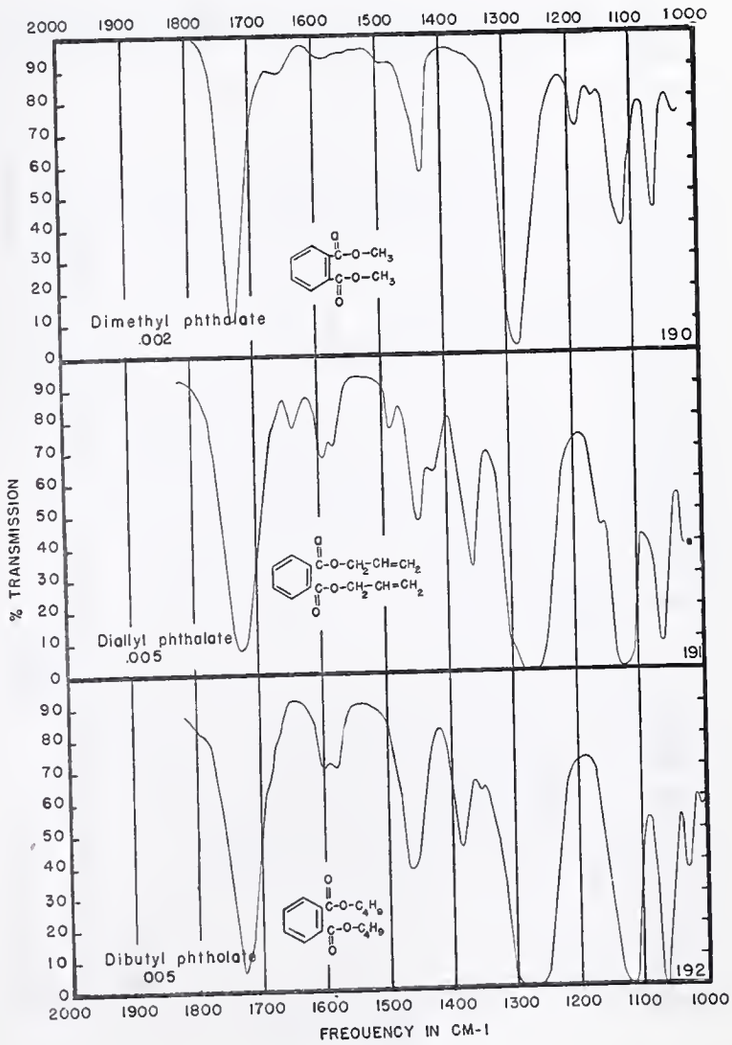

Methyl laurate
.02
175
Methyl oleate
.02
176
Methyl abietate
.02
177
% TRANSMISSION
FREQUENCY IN CM-1

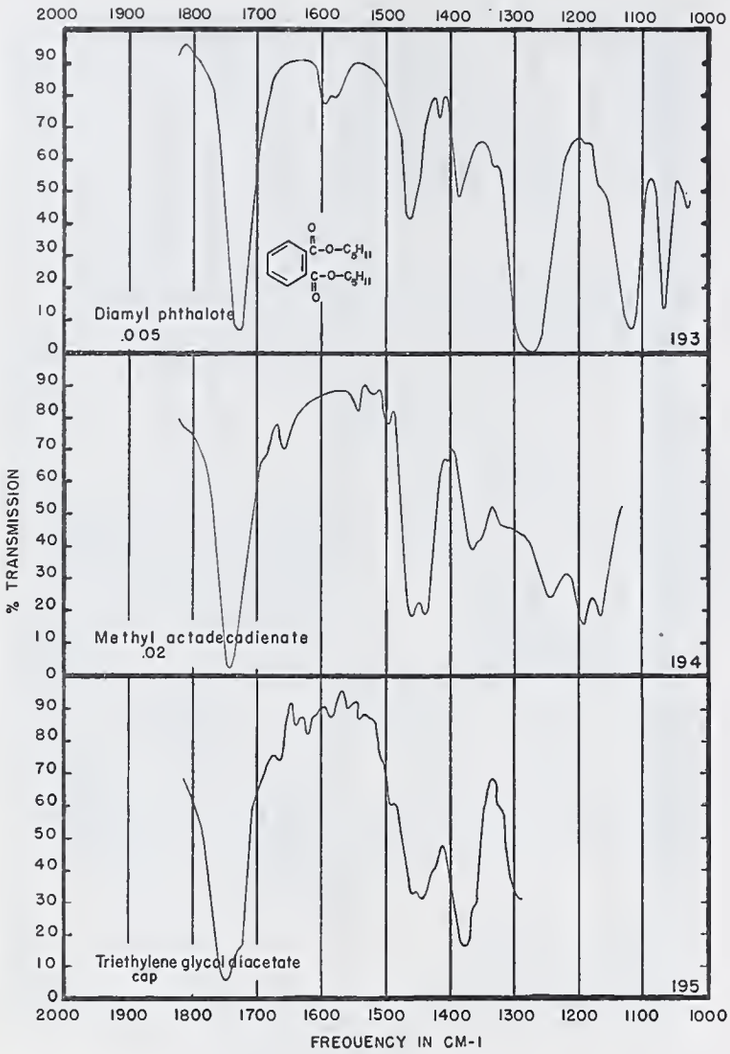

Acetoacetic ester
178
Dimethyl maleate
.005
179
Diethyl maleate
cap
180
% TRANSMISSION
FREQUENCY IN CM-1

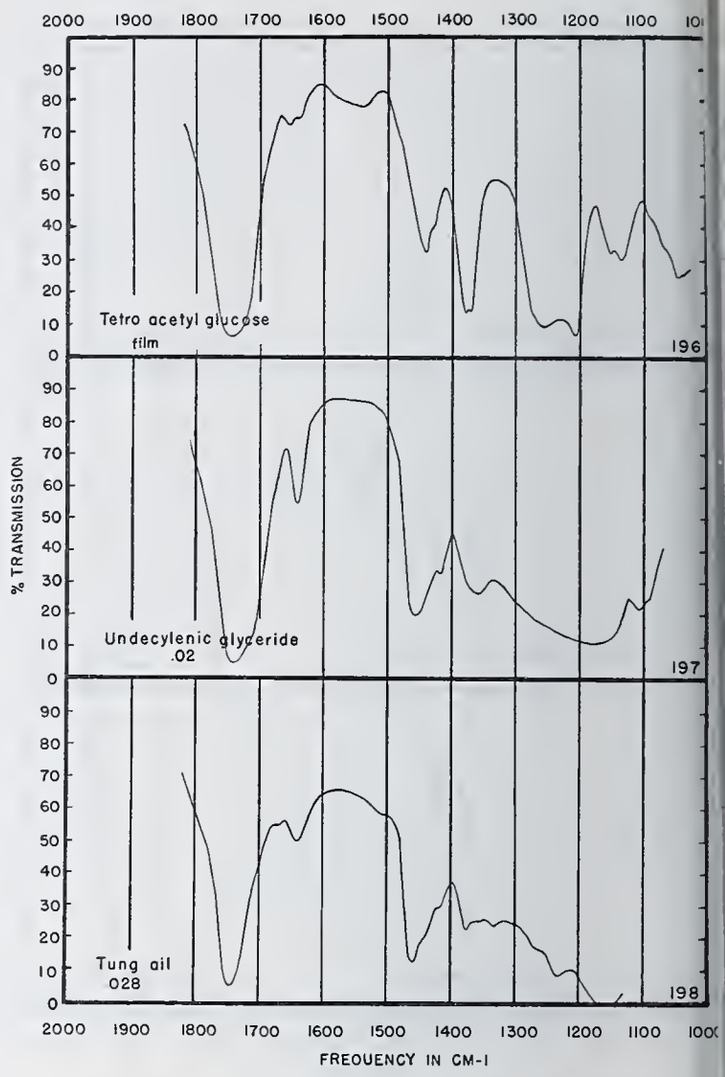

Dibutyl maleate
cap
181
Hexaethylene glycol maleate
film
182
Octadecanediol maleate
film
183
FREQUENCY IN CM-1

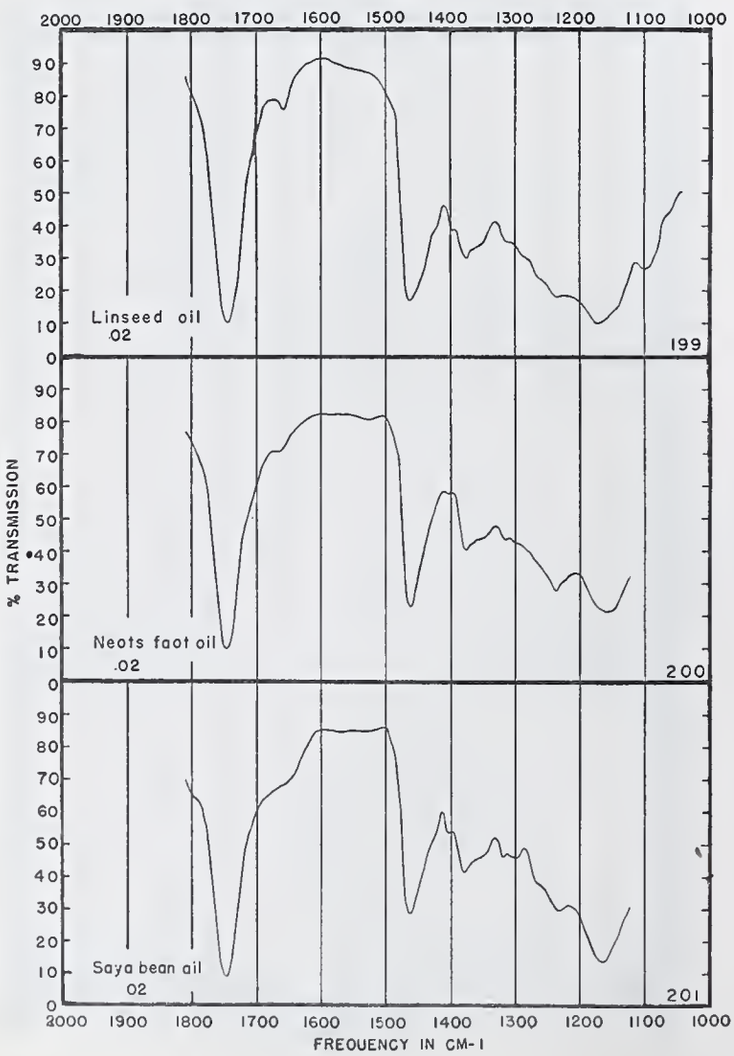

Dimethyl fumarate
film
184
Diethyl fumarate
.005
185
Dibutyl fumarate
.005
186
% TRANSMISSION
FREQUENCY IN CM-1

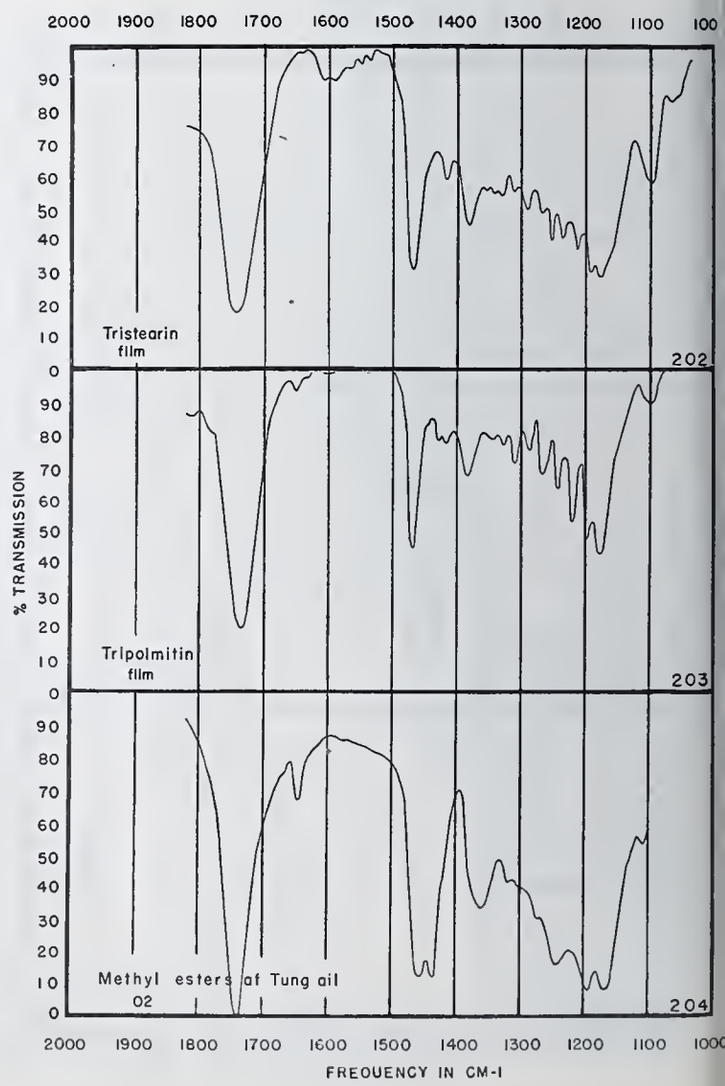

Dimethyl itaconate
.0023
187
Diallyl adipate
.005
188
Diallyl sebacate
.005
189
% TRANSMISSION
FREQUENCY IN CM-1

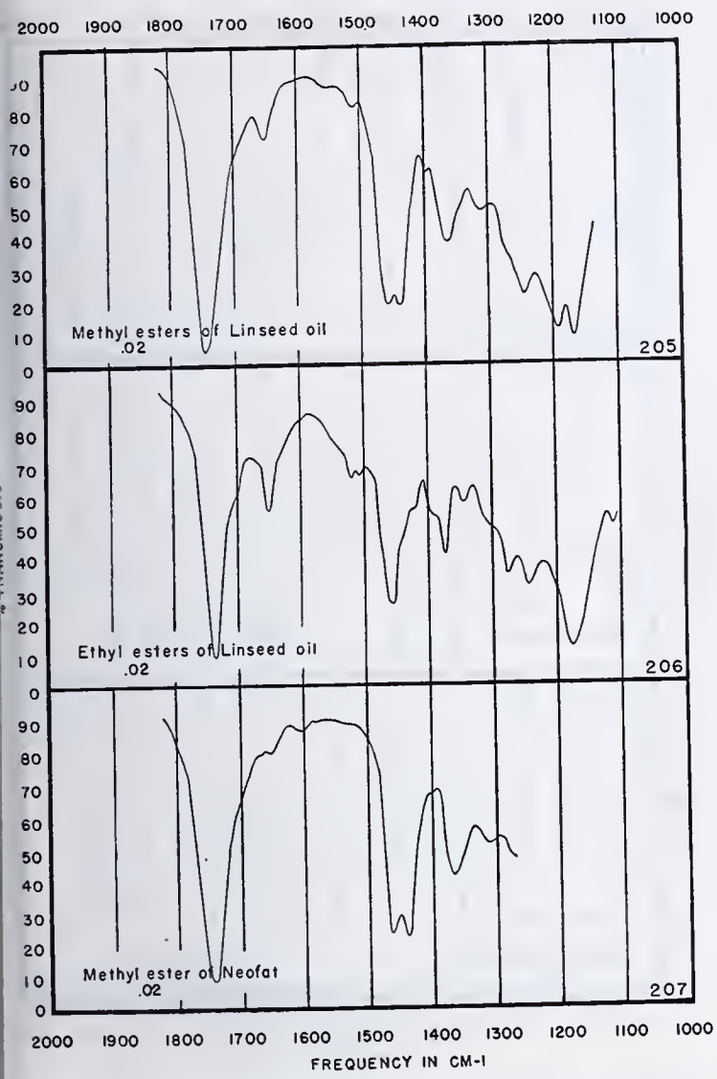

Dimethyl phthalate
.002
190
Diallyl phthalate
.005
191
Dibutyl phthalate
005
192
% TRANSMISSION
FREQUENCY IN CM-1

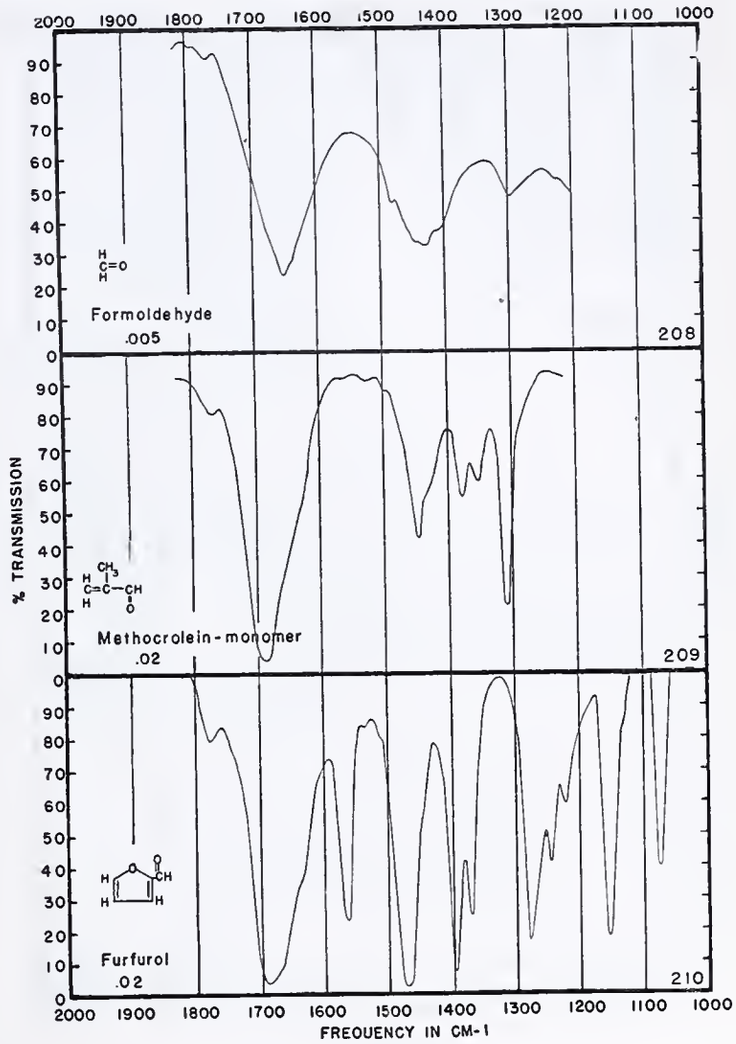

Diamyl phthalate
.005
193
Methyl actadecadienate
.02
194
Triethylene glycol diacetate
cap
195
% TRANSMISSION
FREQUENCY IN CM-1

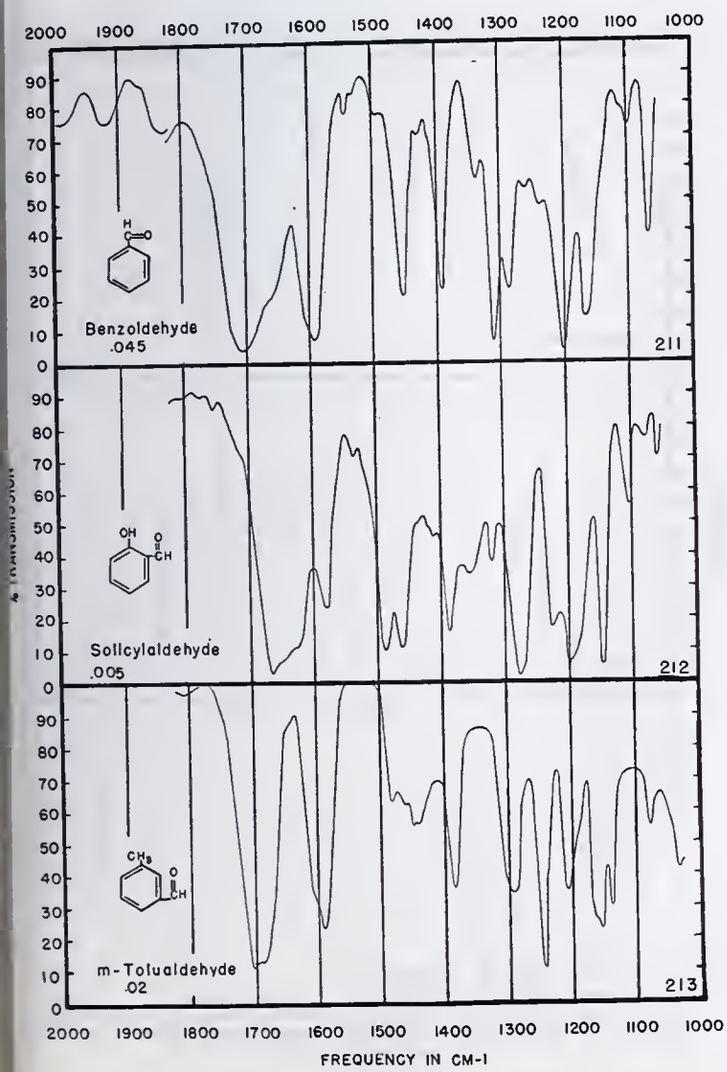

Tetra acetyl glucose
film
196
Undecylenic glyceride
.02
197
Tung oil
.028
198
% TRANSMISSION
FREQUENCY IN CM-1

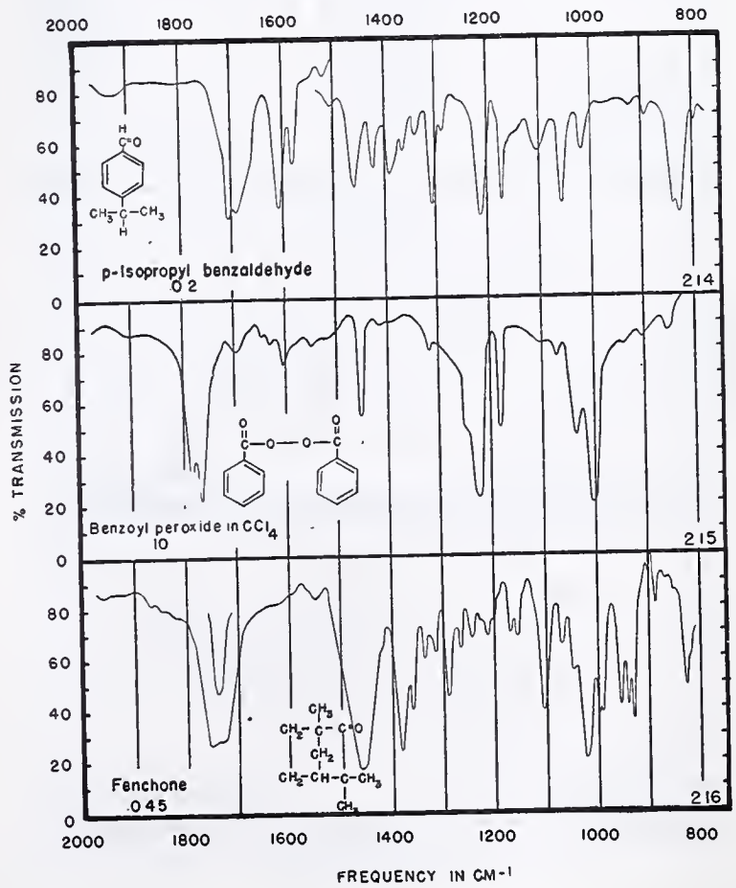

Linseed oil
.02
199
Neots foot oil
.02
200
Soya bean oil
02
201
% TRANSMISSION
FREQUENCY IN CM-1

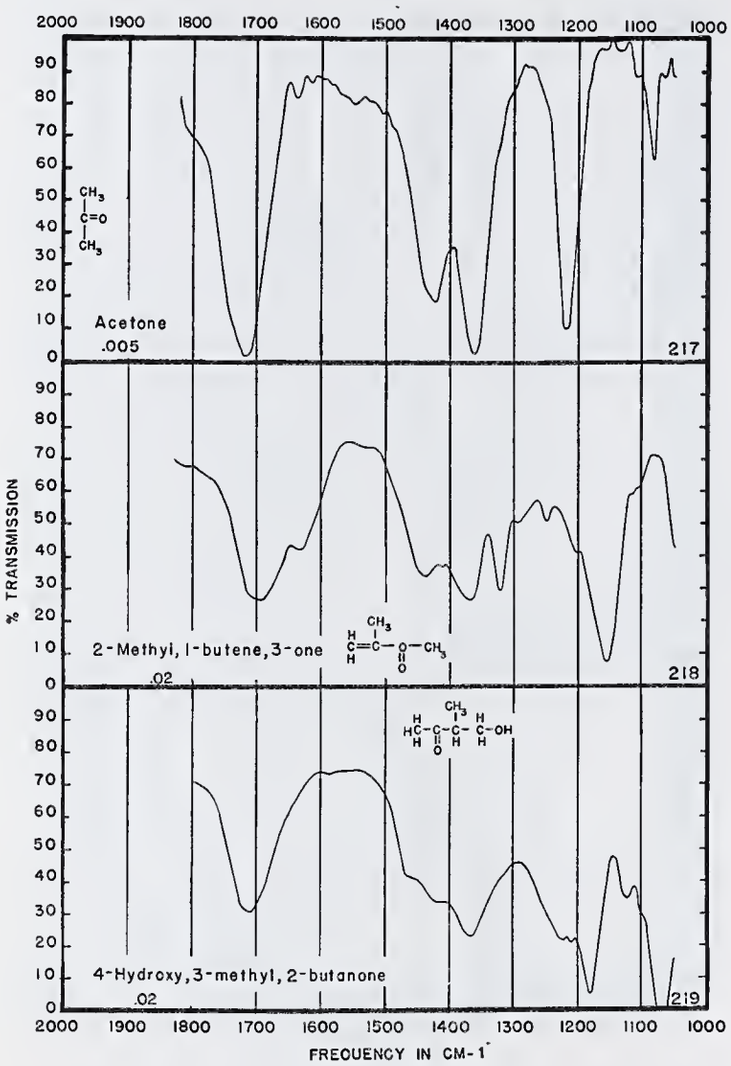

Tristearin
film
202
Tripolmitin
film
203
Methyl esters of Tung oil
02
204
% TRANSMISSION
FREQUENCY IN CM-1

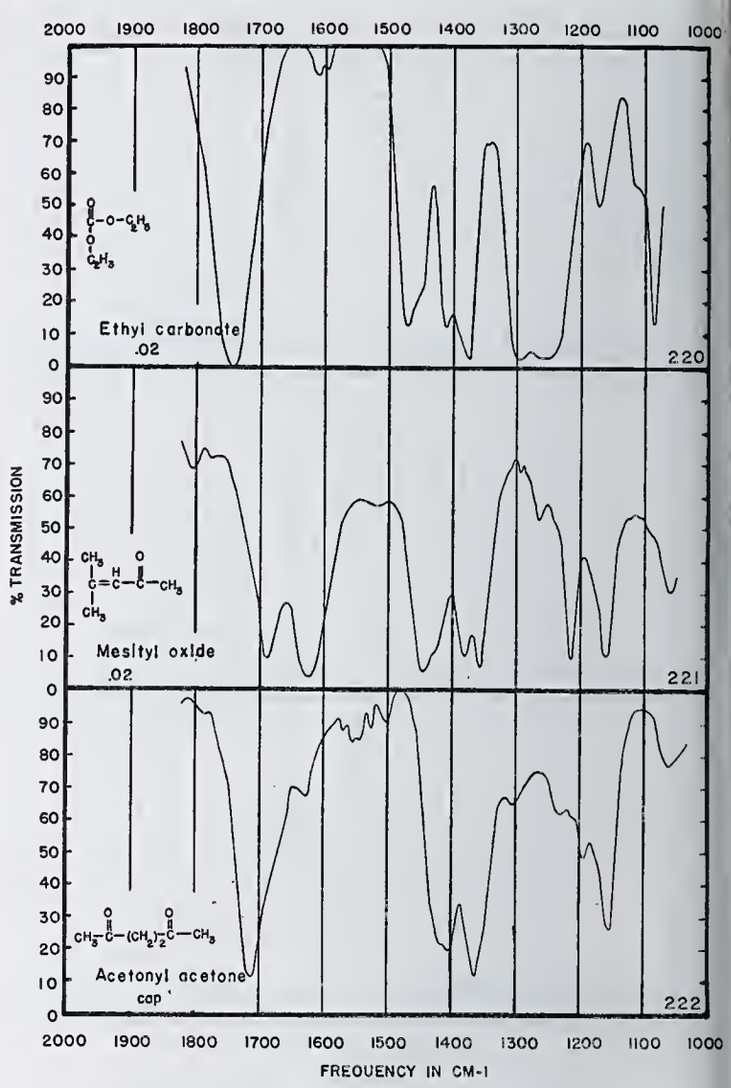

Methyl esters of Linseed oil
.02
205
Ethyl esters of Linseed oil
.02
206
Methyl ester of Neofat
.02
207
FREQUENCY IN CM-1

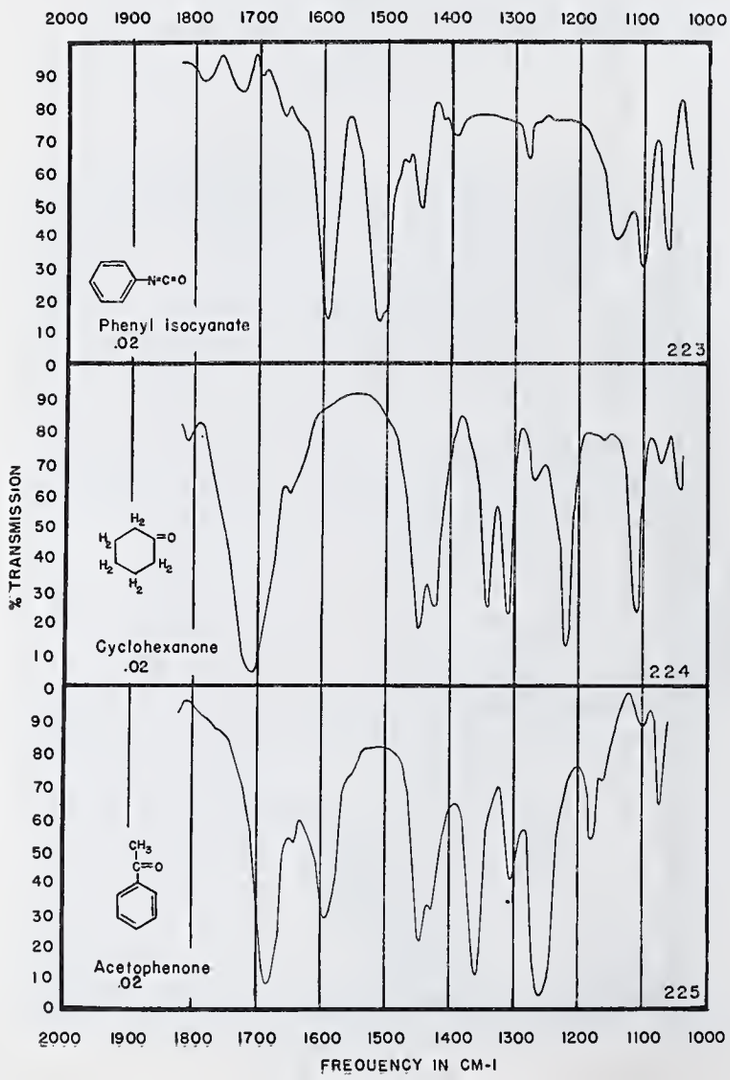

% TRANSMISSION
Formoldehyde
.005
208
Methocrolein-monomer
.02
209
Furfurol
.02
210
FREQUENCY IN CM-1

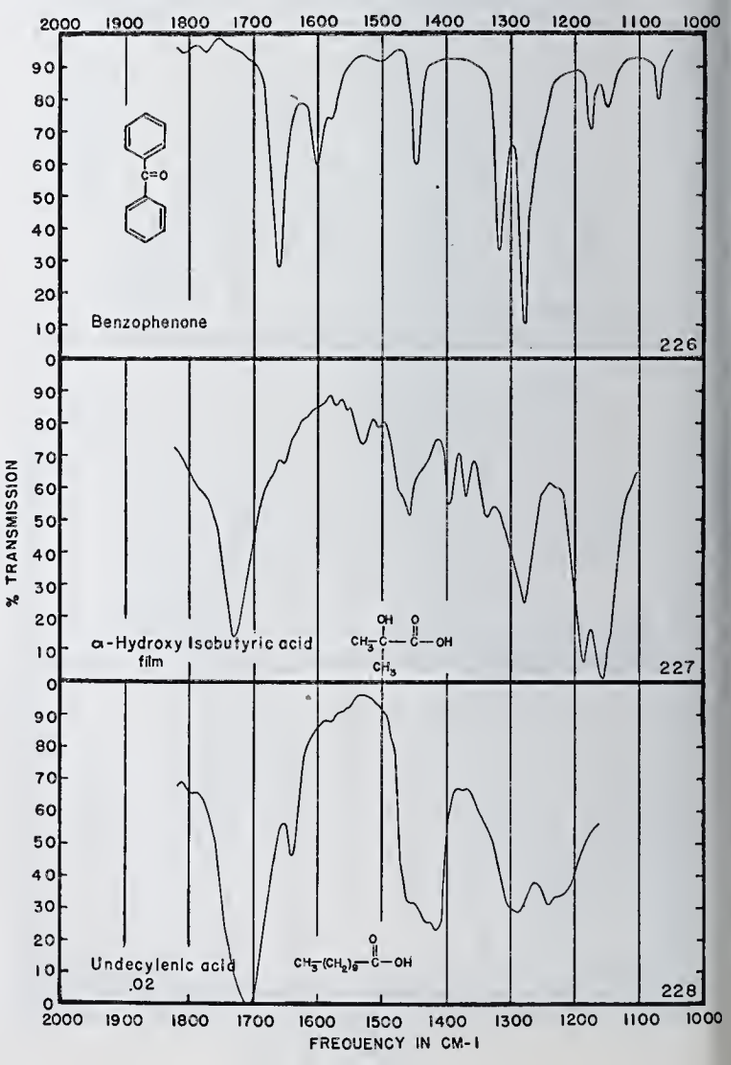

Benzoldehyde
.045
211
Sollcylaldehyde
.005
212
m-Tolualdehyde
.02
213
FREQUENCY IN CM-1

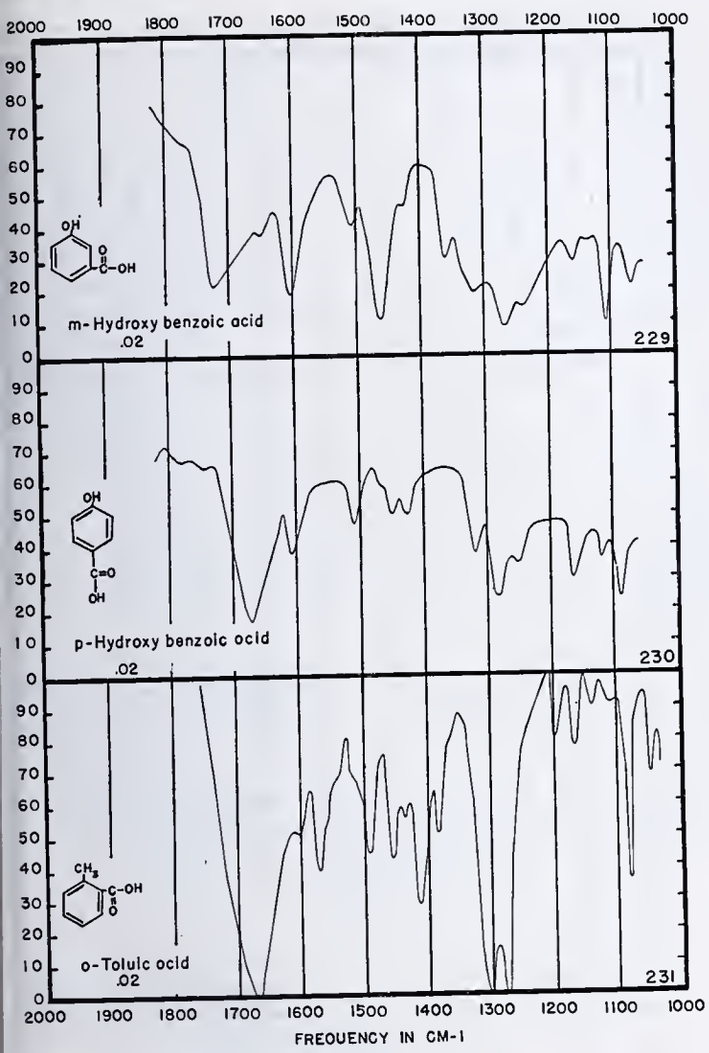

% TRANSMISSION
p-isopropyl benzaldehyde
.02
214
Benzoyl peroxide in CCl_4
.10
215
Fenchone
.045
216
FREQUENCY IN CM-1

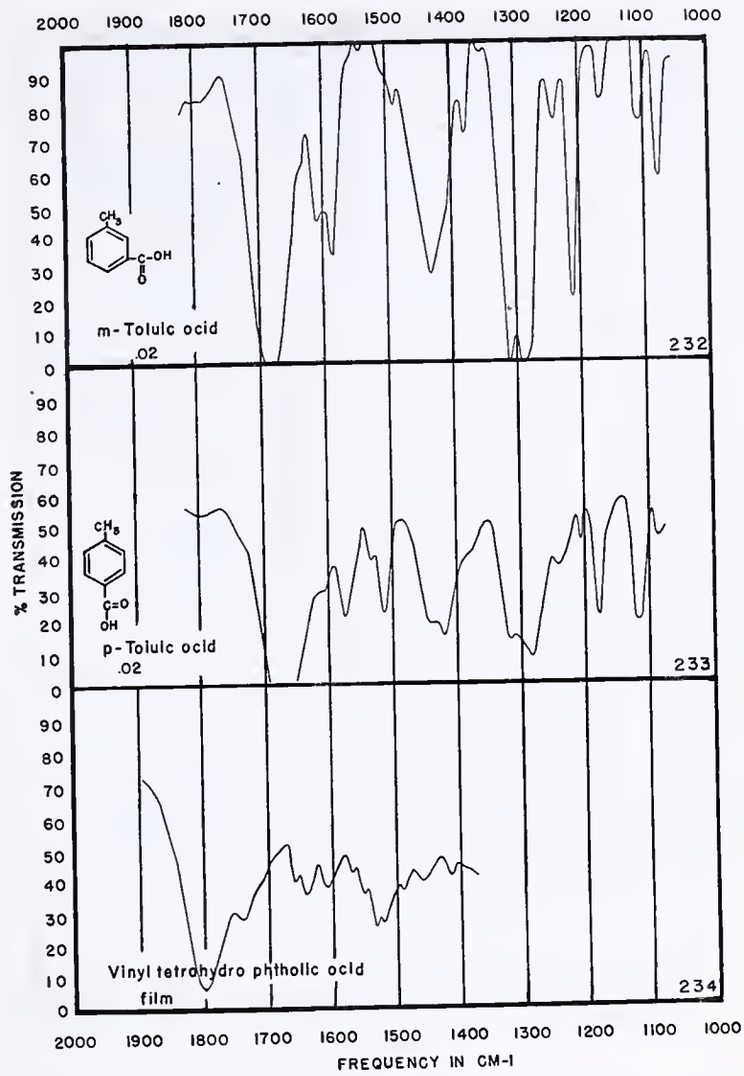

Acetone
.005
217
2-Methyl, 1-butene, 3-one
.02
218
4-Hydroxy, 3-methyl, 2-butanone
.02
219
% TRANSMISSION
FREQUENCY IN CM-1

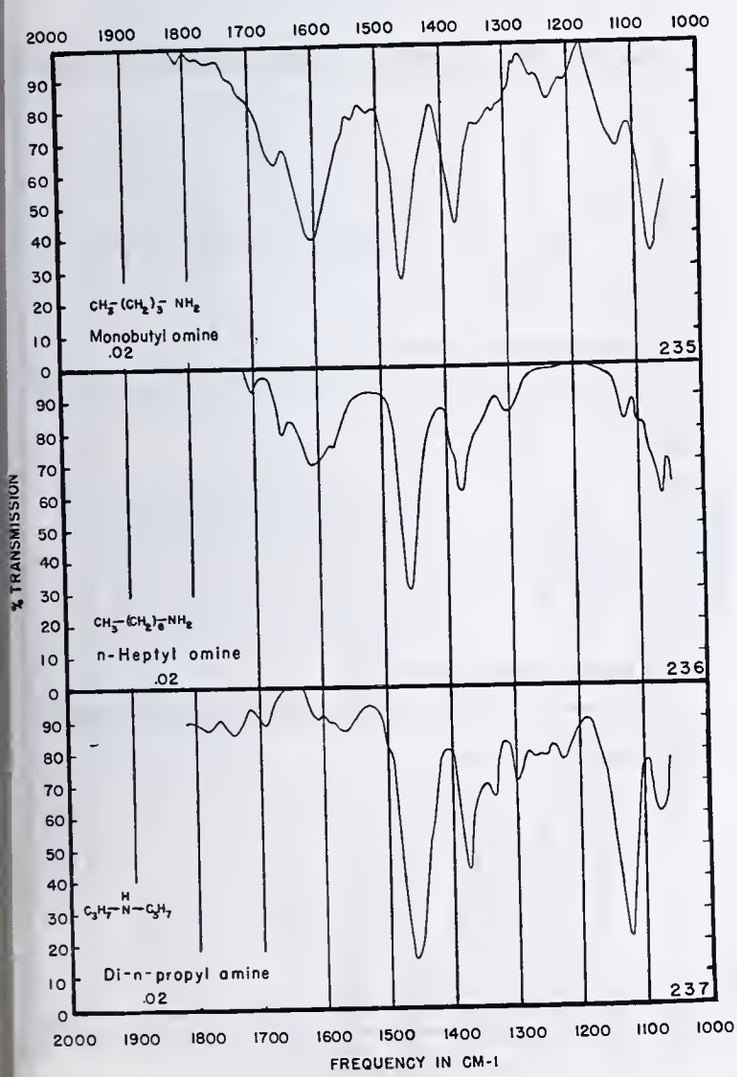

Ethyl carbonate
.02
220
Mesityl oxide
.02
221
Acetonyl acetone
cap
222
% TRANSMISSION
FREQUENCY IN CM-1

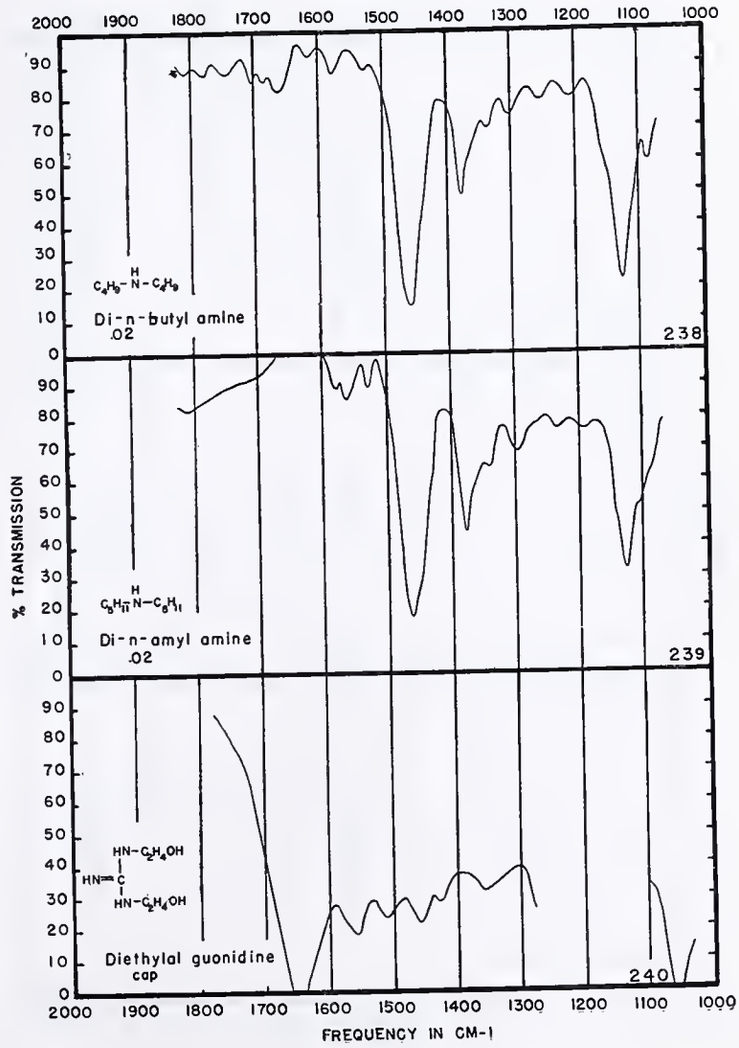

Phenyl isocyanate
.02
223
Cyclohexanone
.02
224
Acetophenone
.02
225
% TRANSMISSION
FREQUENCY IN CM-1

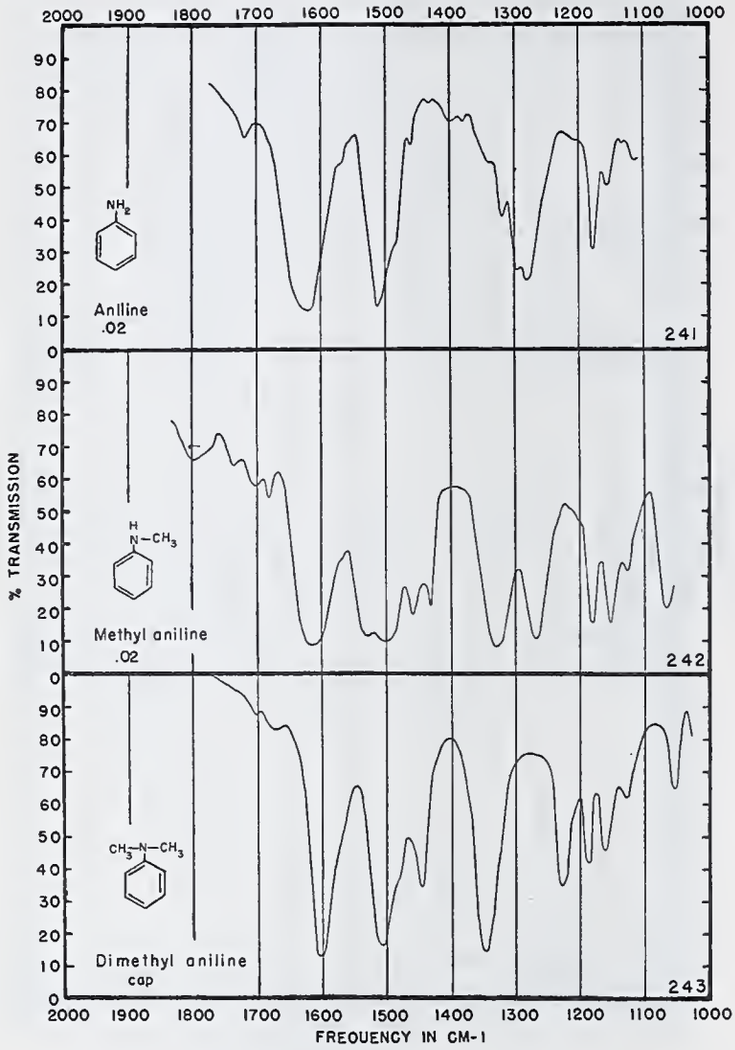

Benzophenone
226
α-Hydroxy Isobutyric acid
film
227
Undecylenic acid
.02
228
% TRANSMISSION
FREQUENCY IN CM-1

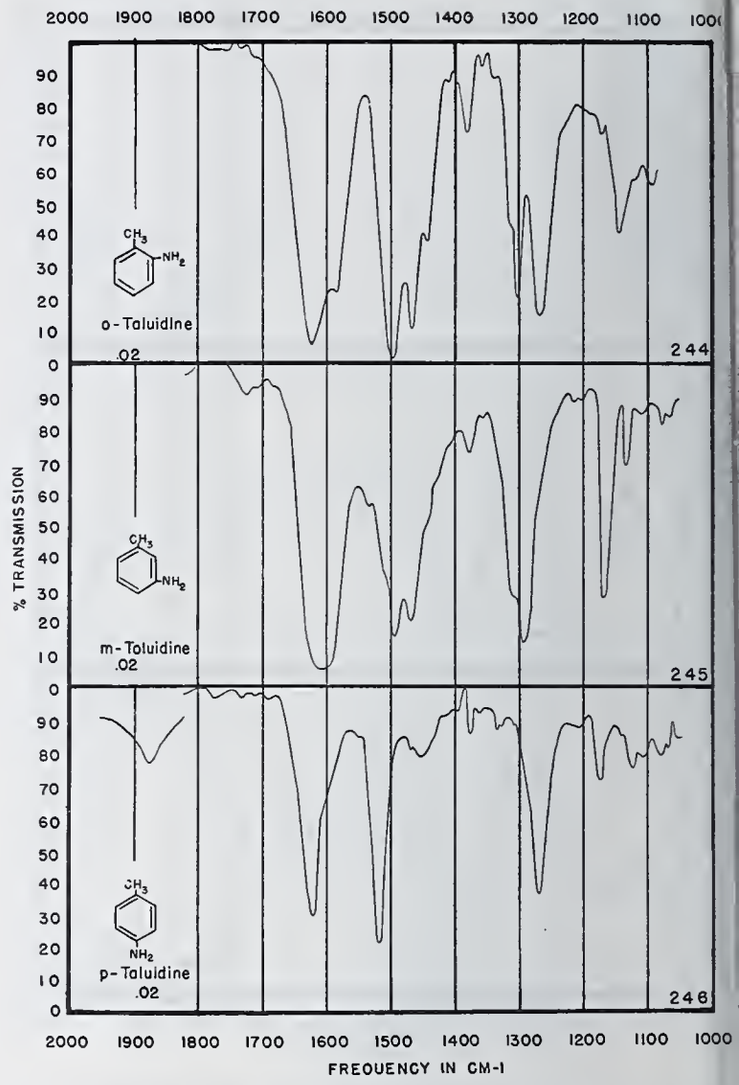

m-Hydroxy benzoic acid
.02
229
p-Hydroxy benzoic acid
.02
230
o-Toluic acid
.02
231
FREQUENCY IN CM-1

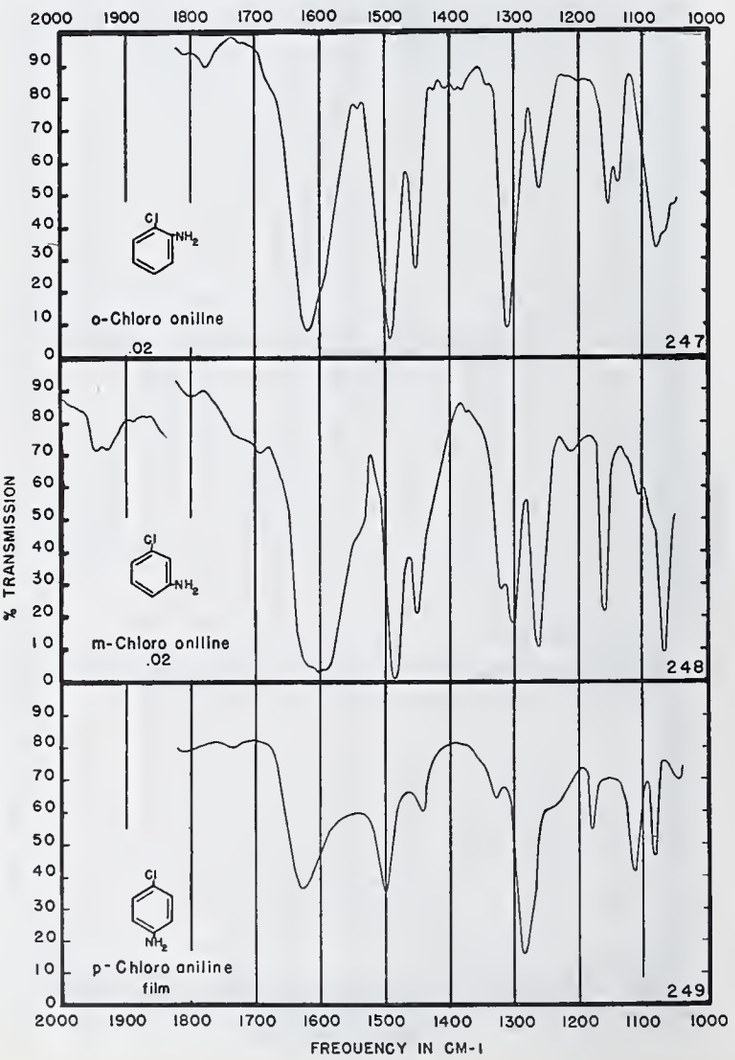

% TRANSMISSION
m-Toluic acid
.02
232
p-Toluic acid
.02
233
Vinyl tetrahydro phthalic acid
film
234
FREQUENCY IN CM-1

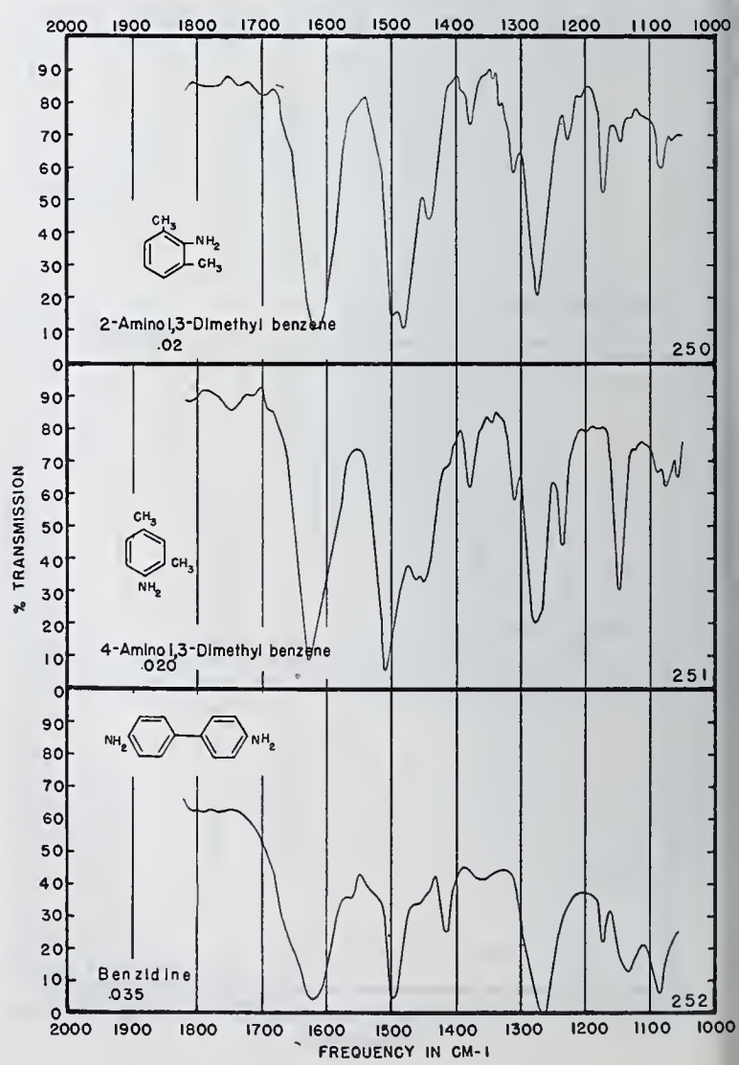

% TRANSMISSION
Monobutyl amine
.02
235
n-Heptyl amine
.02
236
Di-n-propyl amine
.02
237
FREQUENCY IN CM-1

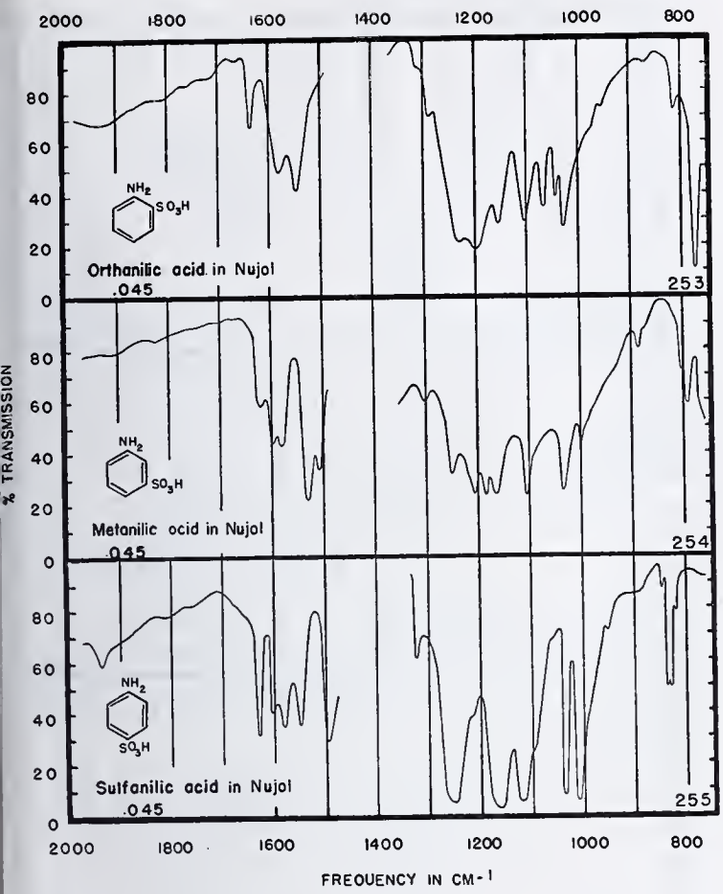

% TRANSMISSION
Di-n-butyl amine
.02
238
Di-n-amyl amine
.02
239
Diethylal guanidine
cap
240
FREQUENCY IN CM-1

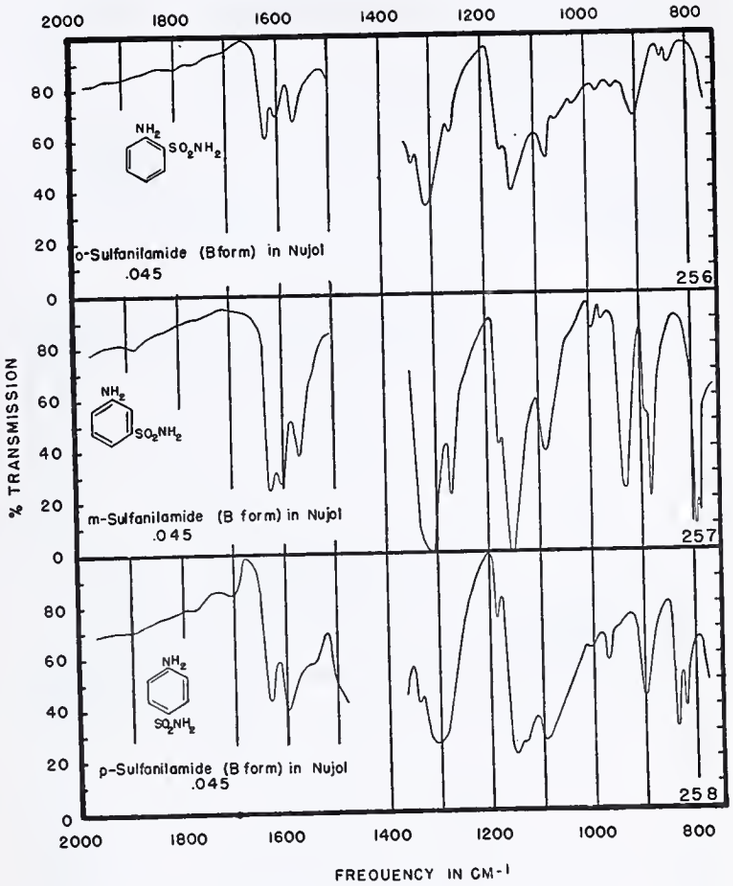

Aniline
.02
241
Methyl aniline
.02
242
Dimethyl aniline
cap
243
% TRANSMISSION
FREQUENCY IN CM-1

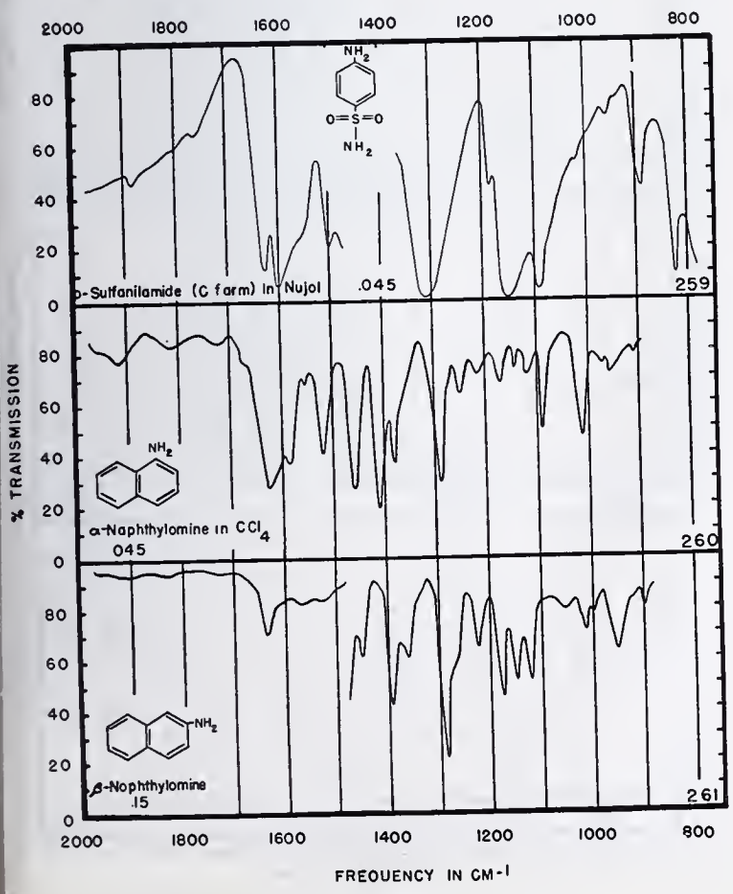

o-Toluidine
.02
244
m-Toluidine
.02
245
p-Toluidine
.02
246
% TRANSMISSION
FREQUENCY IN CM-1

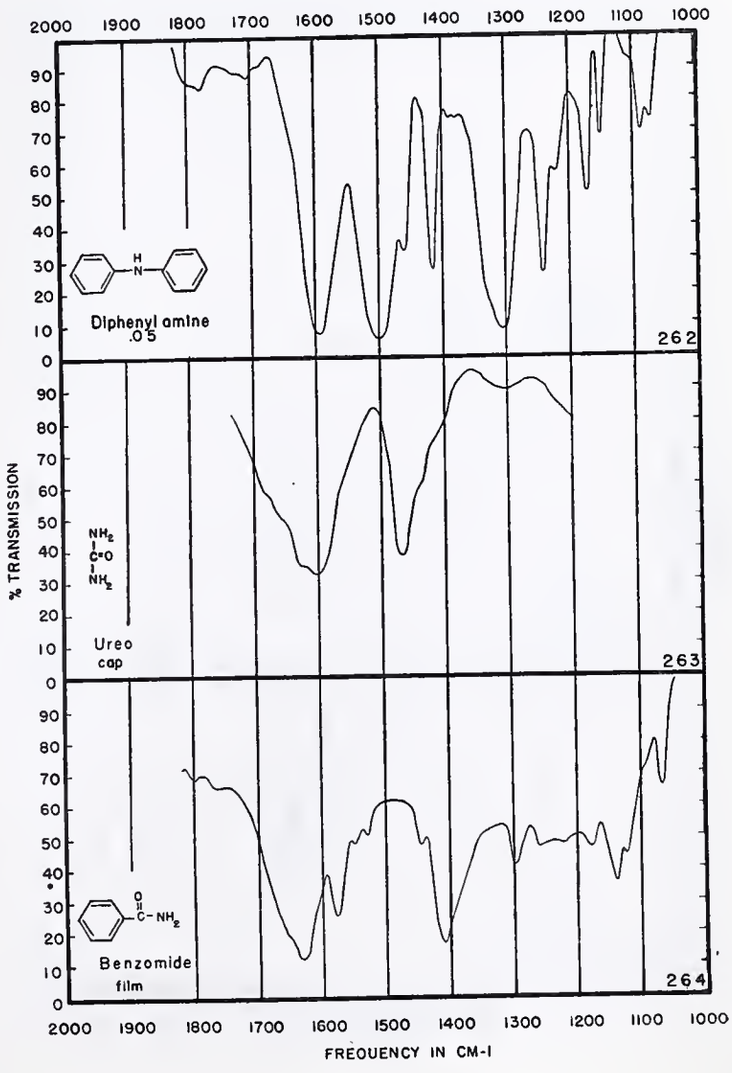

o-Chloro aniline
.02
247
m-Chloro aniline
.02
248
p-Chloro aniline
film
249
% TRANSMISSION
FREQUENCY IN CM-1

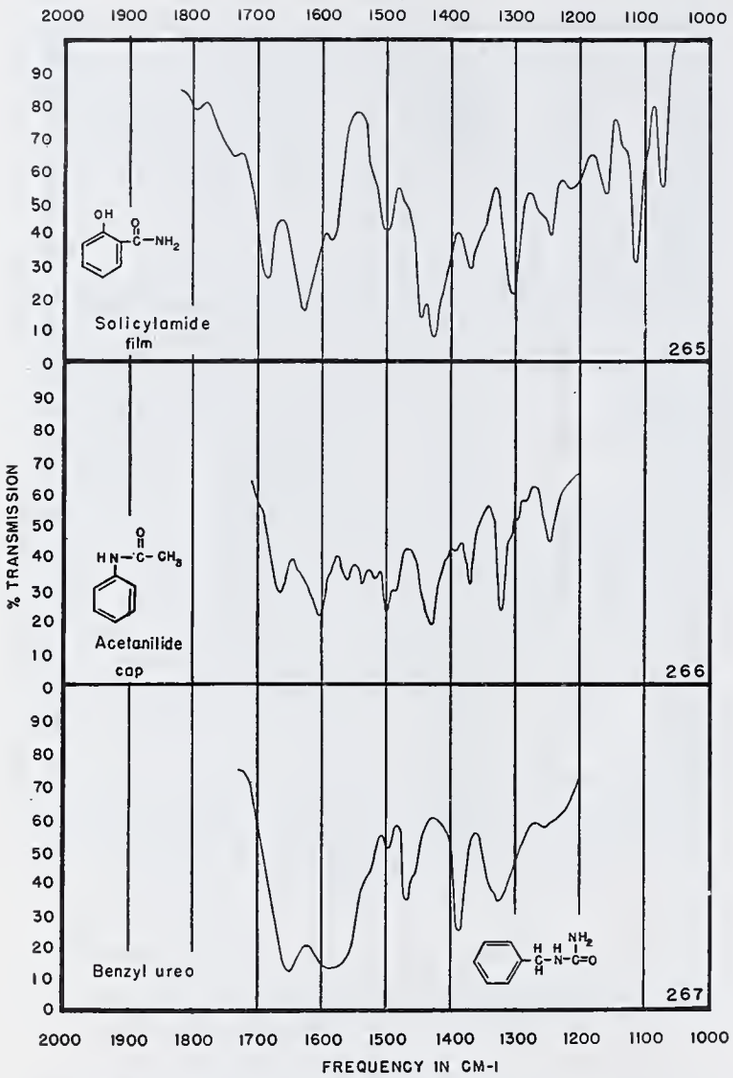

2-Amino 1,3-Dimethyl benzene
.02
250
4-Amino 1,3-Dimethyl benzene
.020
251
Benzidine
.035
252
% TRANSMISSION
FREQUENCY IN CM-1

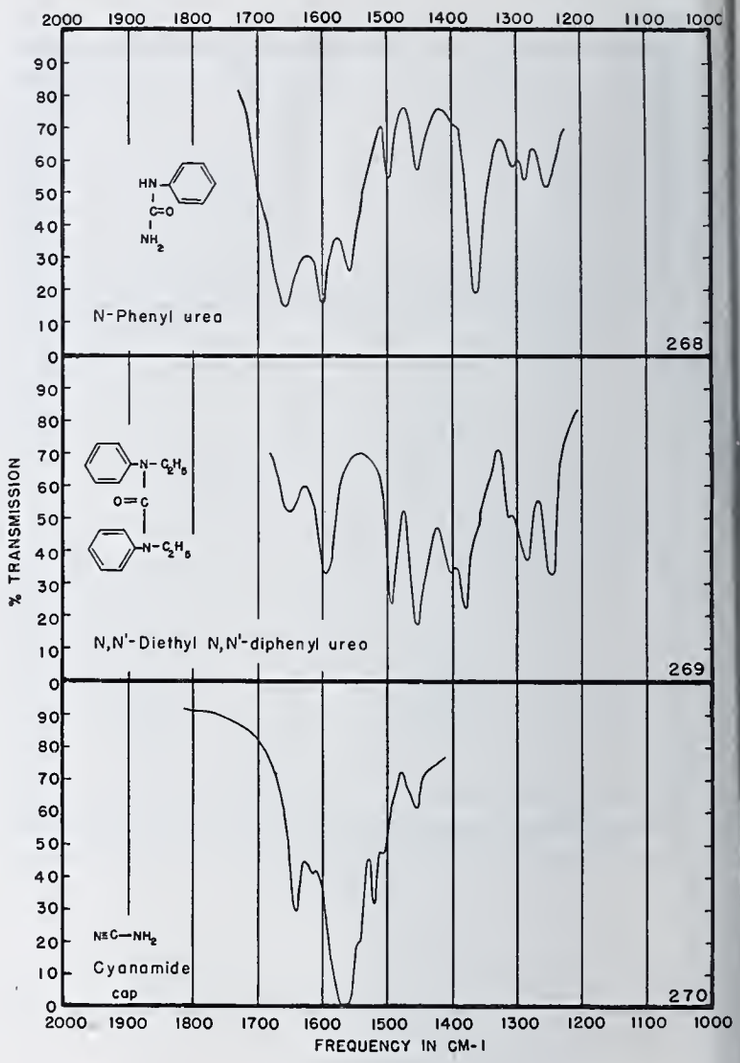

Orthanilic acid in Nujol
.045
253
Metanilic acid in Nujol
.045
254
Sulfanilic acid in Nujol
.045
255
% TRANSMISSION
FREQUENCY IN CM-1

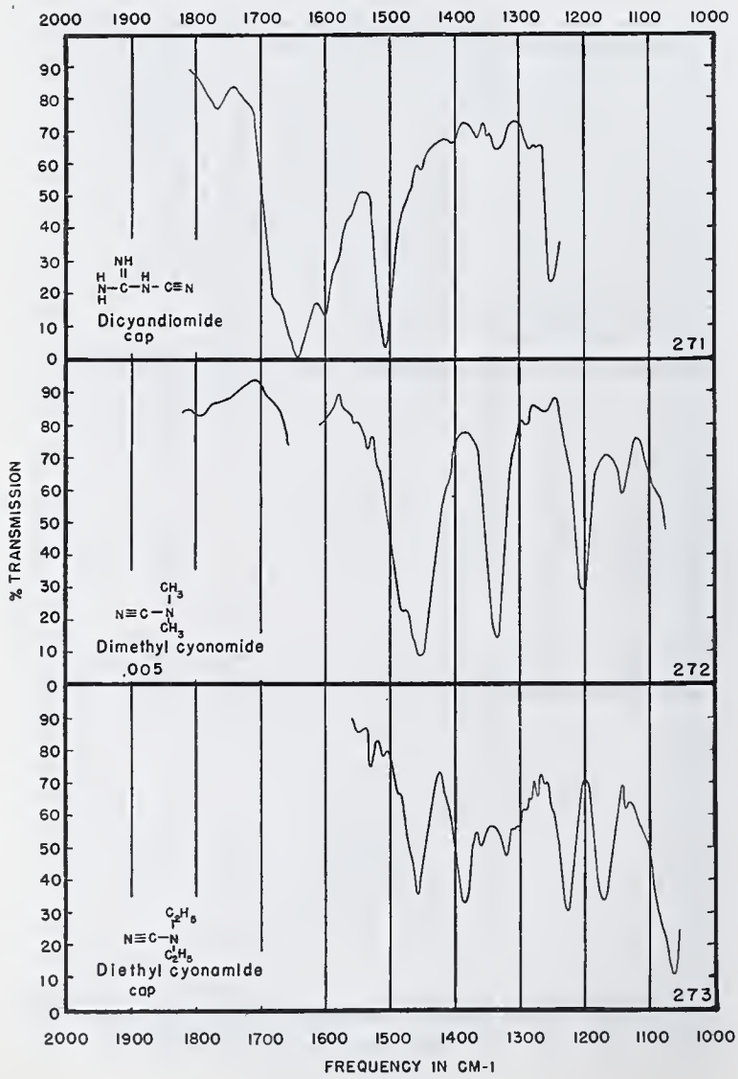

o-Sulfanilamide (B form) in Nujol
.045
256
m-Sulfanilamide (B form) in Nujol
.045
257
p-Sulfanilamide (B form) in Nujol
.045
258
% TRANSMISSION
FREQUENCY IN CM-1

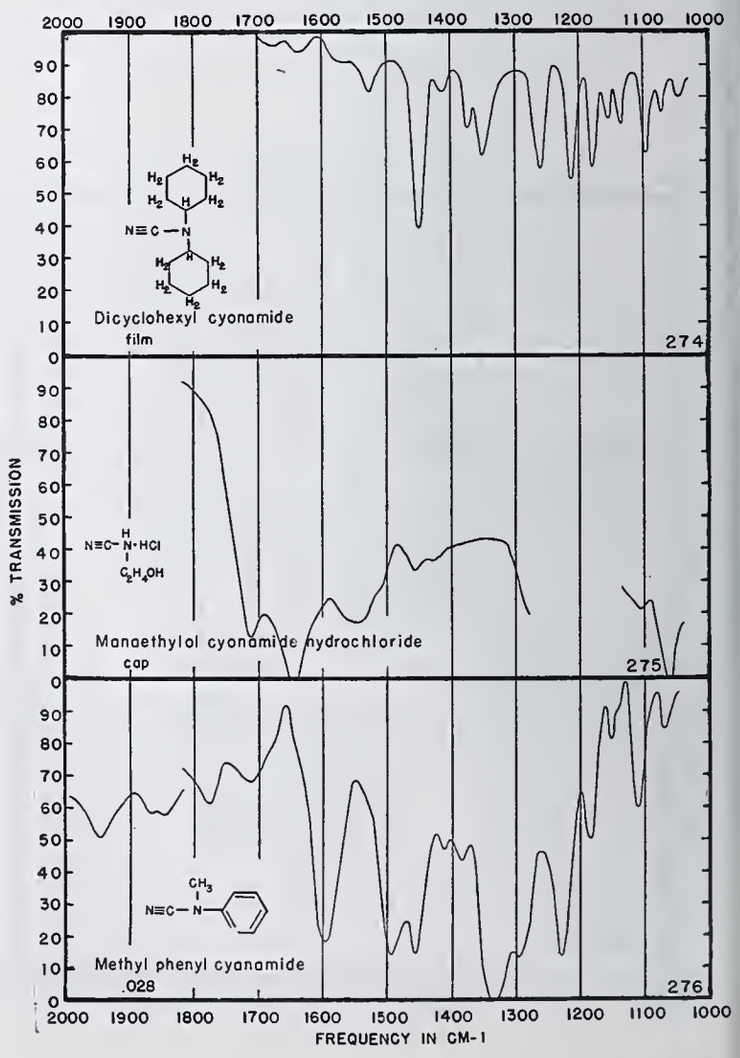

o-Sulfanilamide (C form) in Nujol
.045
259
α-Naphthylamine in CCl4
.045
260
β-Naphthylamine
.15
261
% TRANSMISSION
FREQUENCY IN CM-1

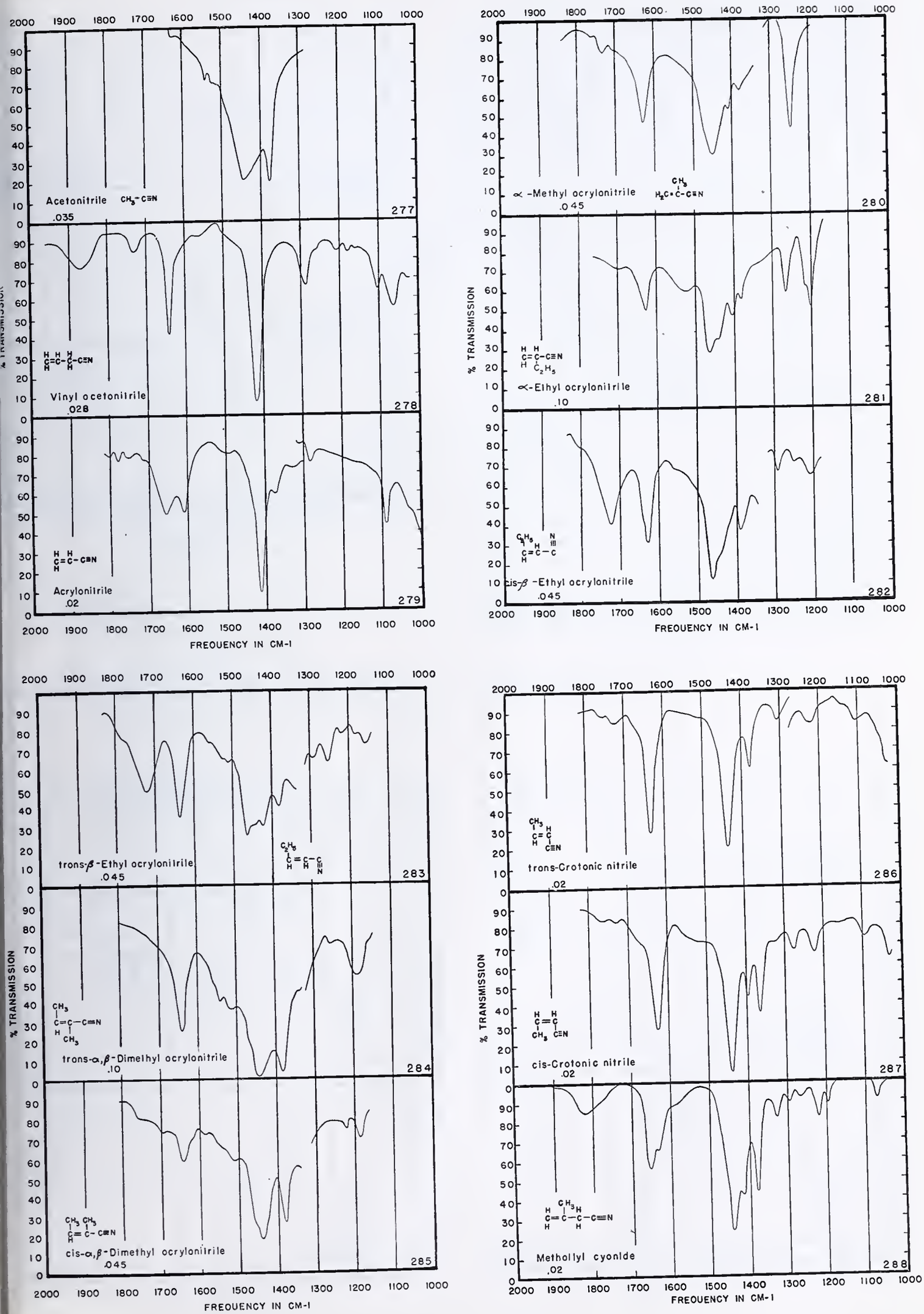

Diphenyl amine
.05
262
Urea
cap
263
Benzamide
film
264
% TRANSMISSION
FREQUENCY IN CM-1

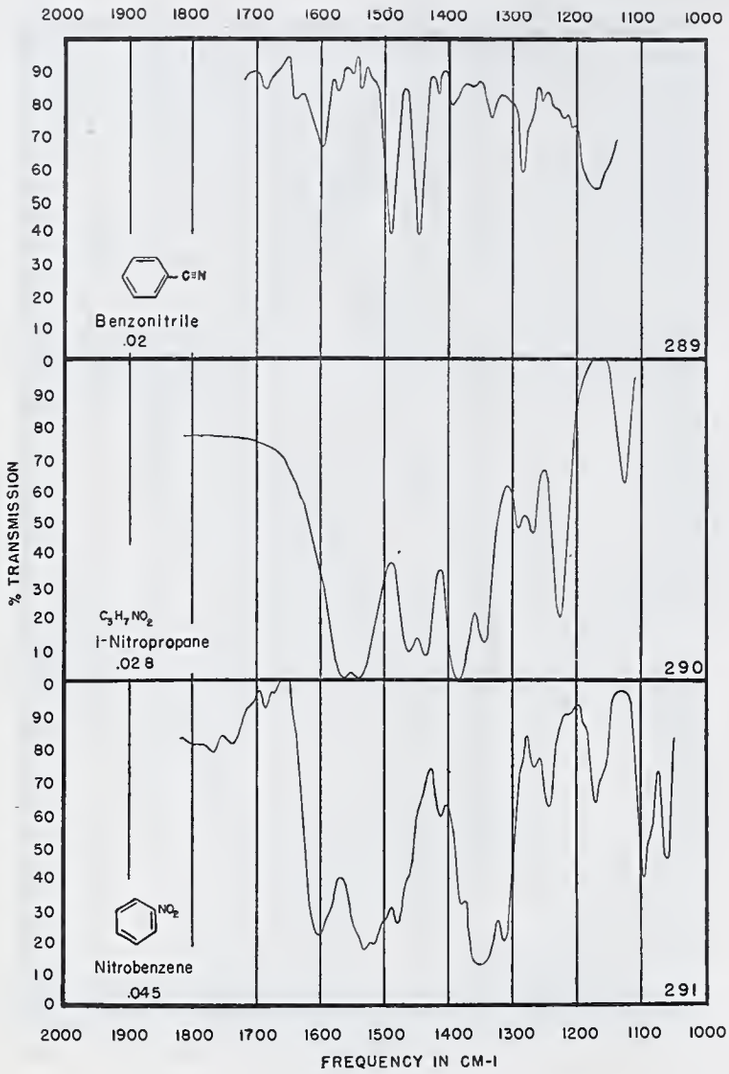

Solicylamide
film
265
Acetanilide
cap
266
Benzyl urea
267
% TRANSMISSION
FREQUENCY IN CM-1

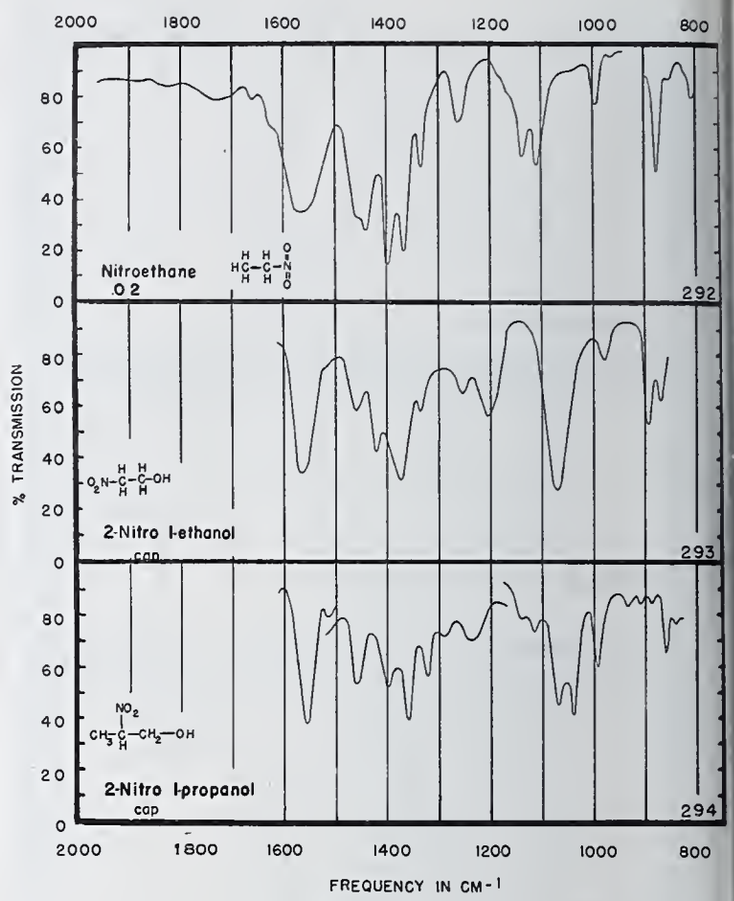

N-Phenyl urea
268
N,N'-Diethyl N,N'-diphenyl urea
269
Cyanamide
cap
270
% TRANSMISSION
FREQUENCY IN CM-1

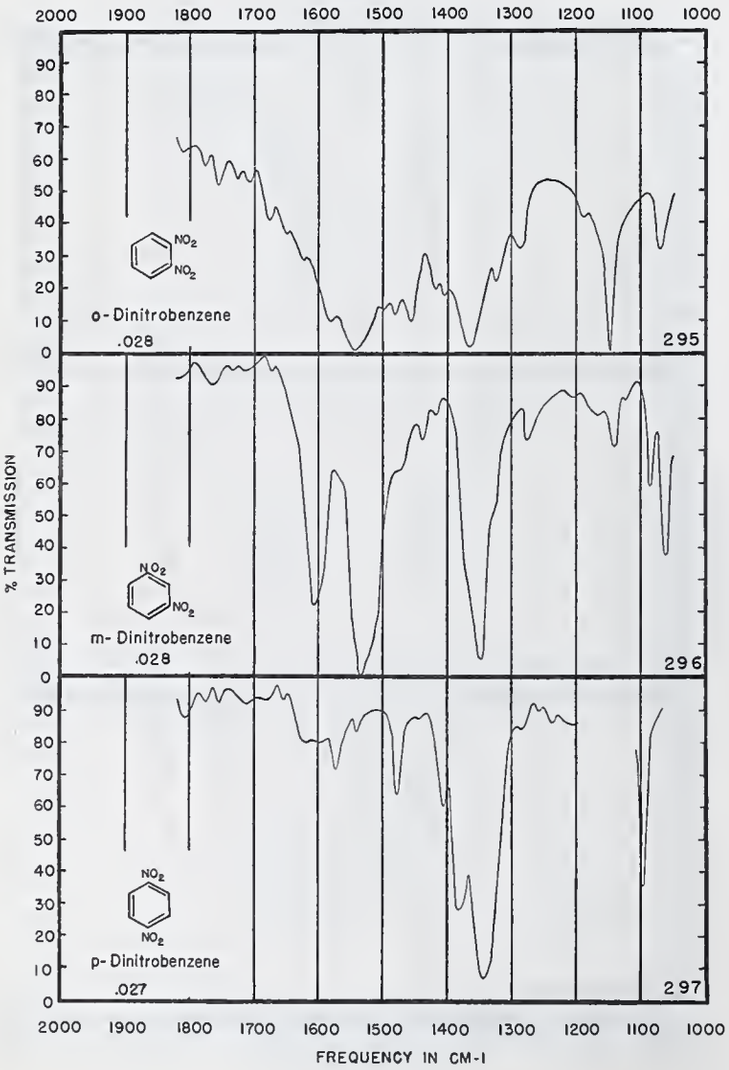

Dicyandiamide
cap
271
Dimethyl cyanamide
.005
272
Diethyl cyanamide
cap
273
% TRANSMISSION
FREQUENCY IN CM-1

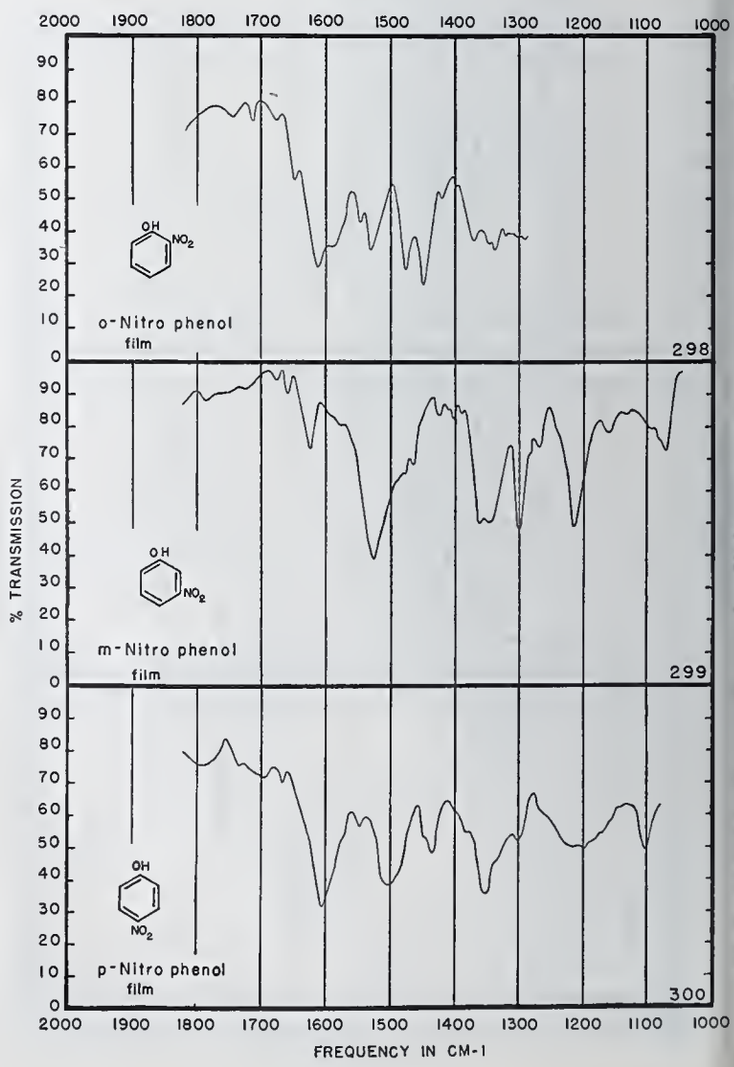

Dicyclohexyl cyanamide
film
274
Monoethylol cyanamide hydrochloride
cap
275
Methyl phenyl cyanamide
.028
276
% TRANSMISSION
FREQUENCY IN CM-1

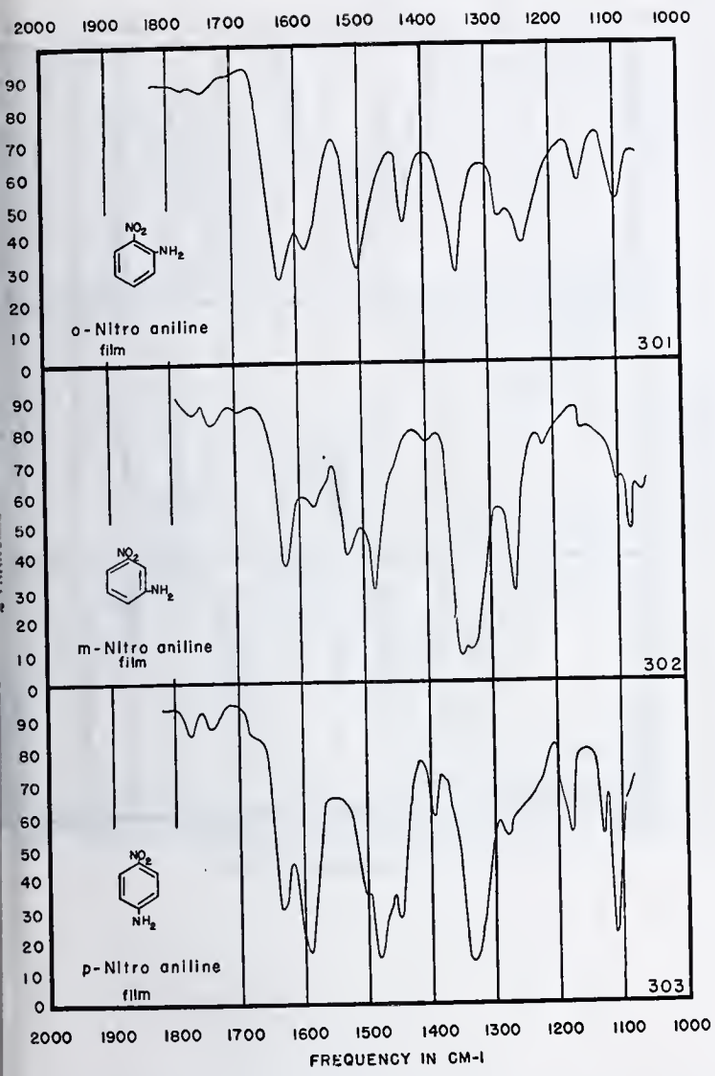

Acetonitrile CH3-C≡N
.035
277
Vinyl acetonitrile
.028
278
Acrylonitrile
.02
279
α-Methyl acrylonitrile
.045
280
α-Ethyl acrylonitrile
.10
281
cis-β-Ethyl acrylonitrile
.045
282
trans-β-Ethyl acrylonitrile
.045
283
trans-α,β-Dimethyl acrylonitrile
.10
284
cis-α,β-Dimethyl acrylonitrile
.045
285
trans-Crotonic nitrile
.02
286
cis-Crotonic nitrile
.02
287
Methallyl cyanide
.02
288
% TRANSMISSION
FREQUENCY IN CM-1

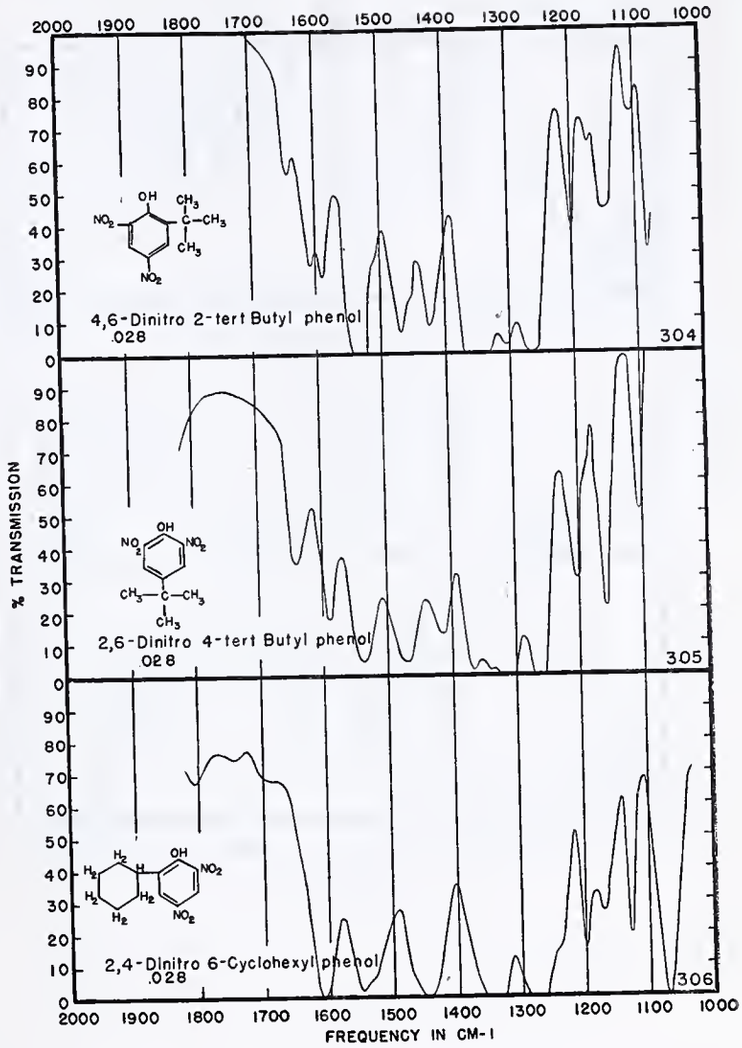

% TRANSMISSION
FREQUENCY IN CM-1
Benzonitrile
.02
289
$C_3H_7NO_2$
1-Nitropropane
.028
290
Nitrobenzene
.045
291

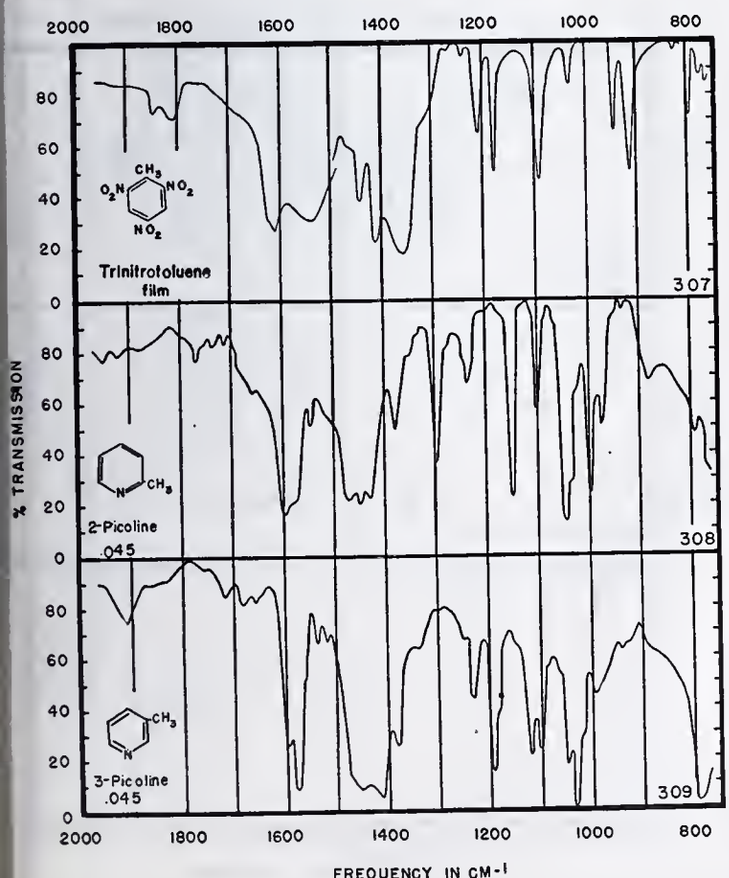

% TRANSMISSION
FREQUENCY IN CM-1
Nitroethane
.02
292
2-Nitro 1-ethanol
cap
293
2-Nitro 1-propanol
cap
294

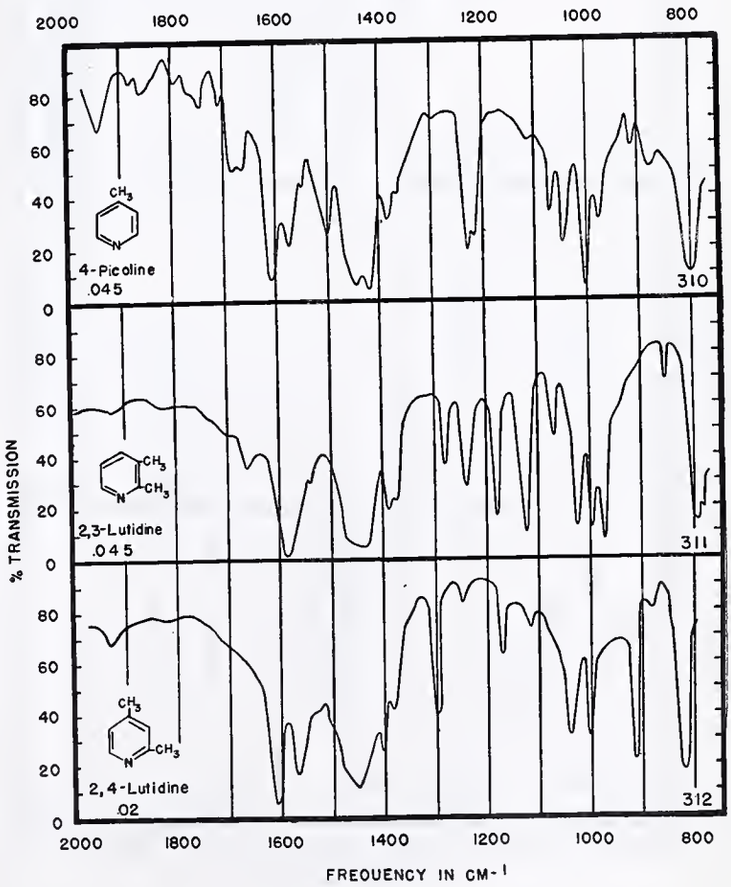

% TRANSMISSION
FREQUENCY IN CM-1
o-Dinitrobenzene
.028
295
m-Dinitrobenzene
.028
296
p-Dinitrobenzene
.027
297

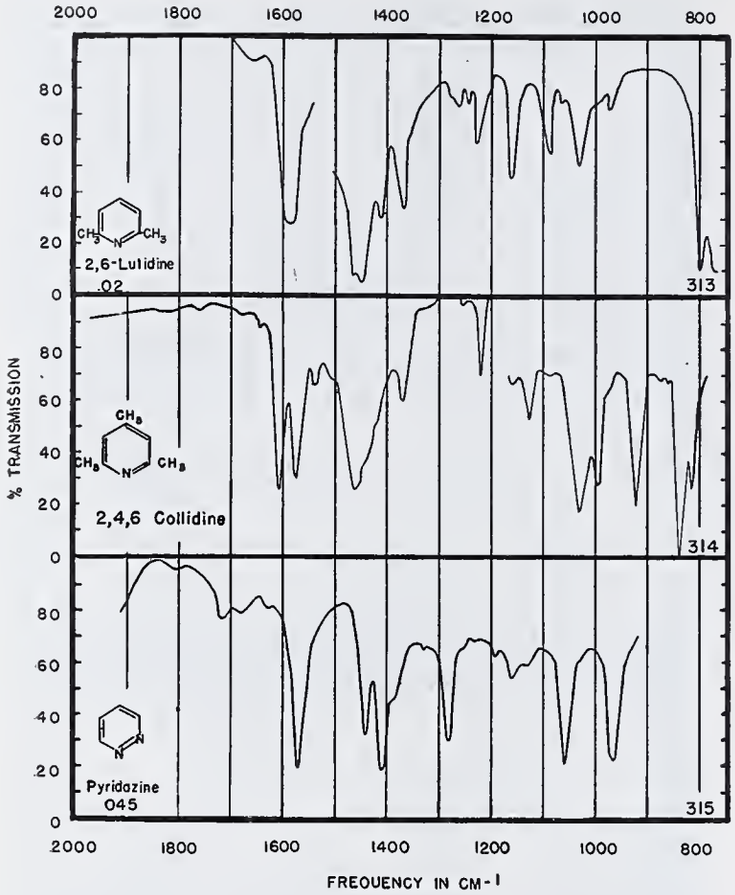

% TRANSMISSION
FREQUENCY IN CM-1
o-Nitro phenol
film
298
m-Nitro phenol
film
299
p-Nitro phenol
film
300

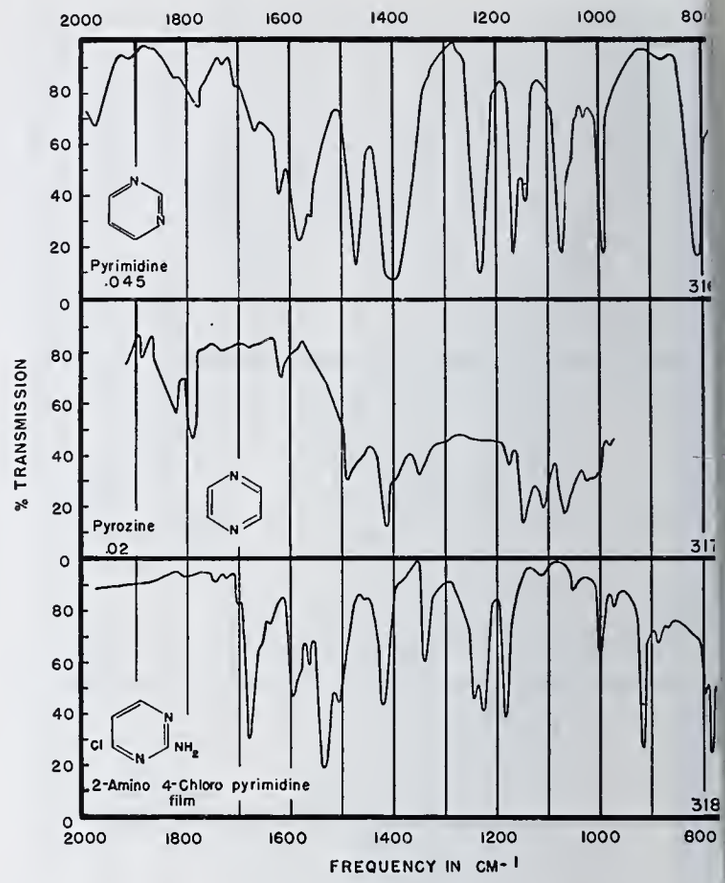

o-Nitro aniline
film
301
m-Nitro aniline
film
302
p-Nitro aniline
film
303
FREQUENCY IN CM-1

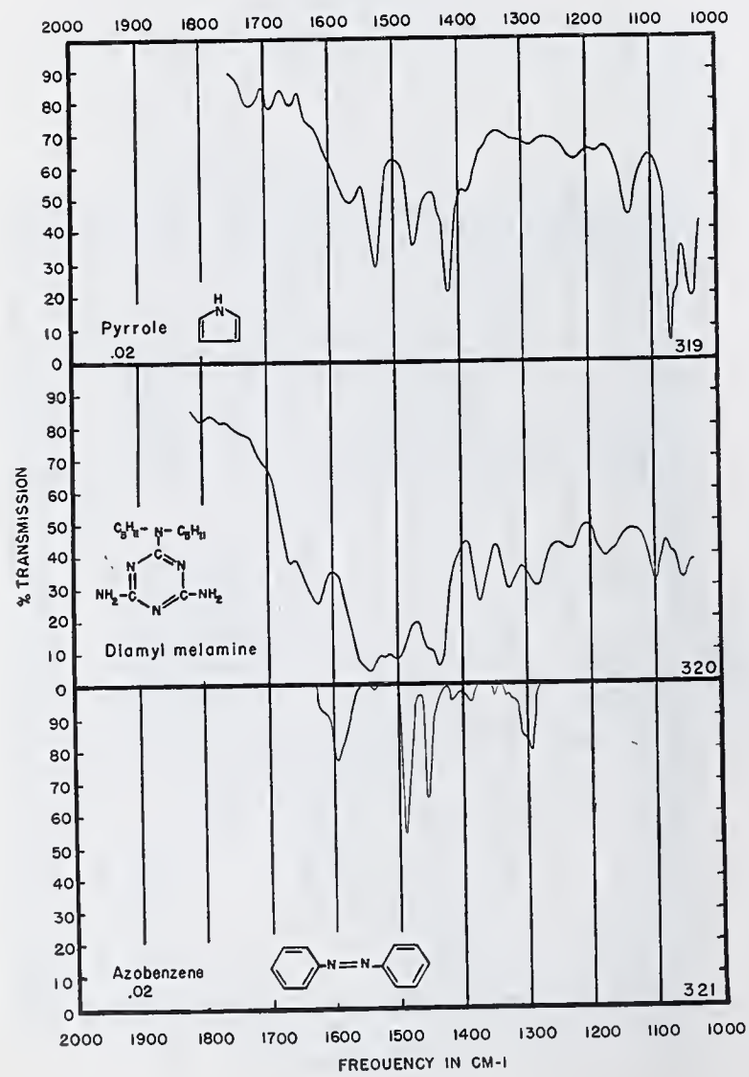

4,6-Dinitro 2-tert Butyl phenol
.028
304
2,6-Dinitro 4-tert Butyl phenol
.028
305
2,4-Dinitro 6-Cyclohexyl phenol
.028
306
% TRANSMISSION
FREQUENCY IN CM-1

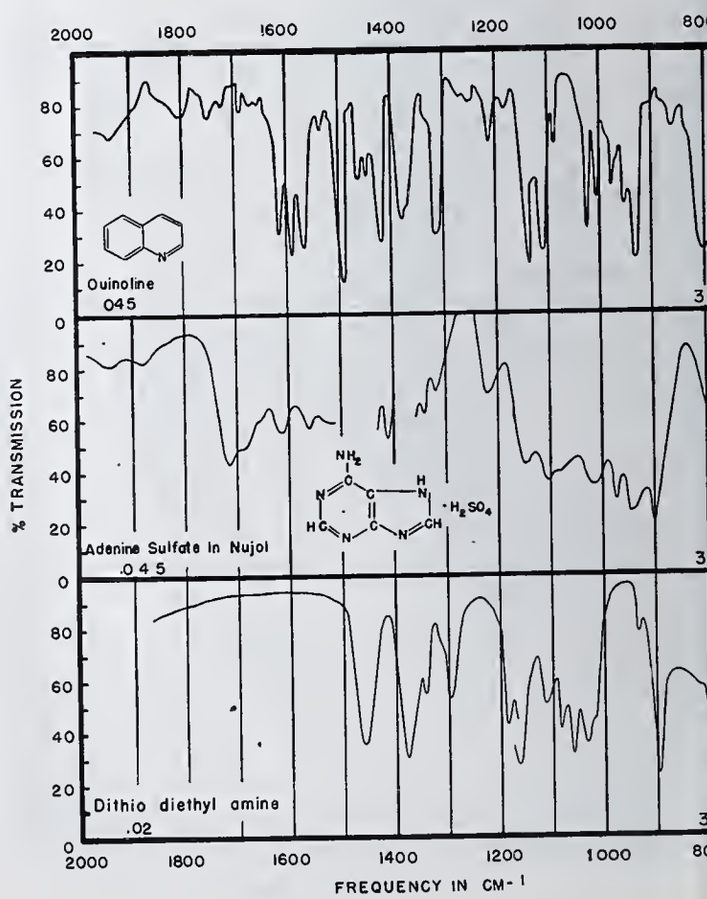

Trinitrotoluene
film
307
2-Picoline
.045
308
3-Picoline
.045
309
% TRANSMISSION
FREQUENCY IN CM-1

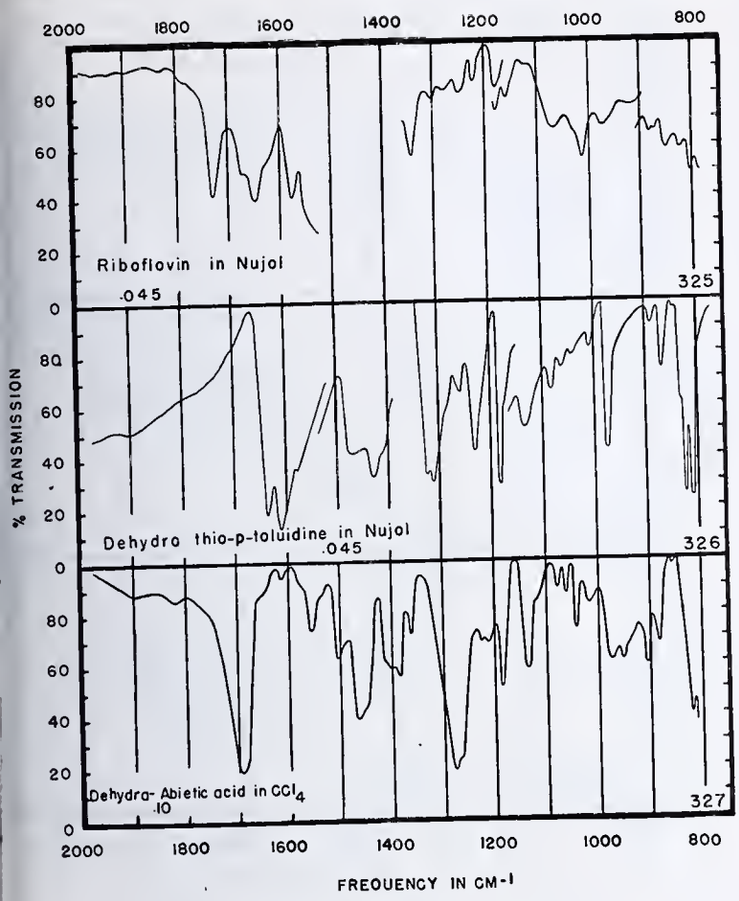

4-Picoline
.045
310
2,3-Lutidine
.045
311
2,4-Lutidine
.02
312
% TRANSMISSION
FREQUENCY IN CM-1

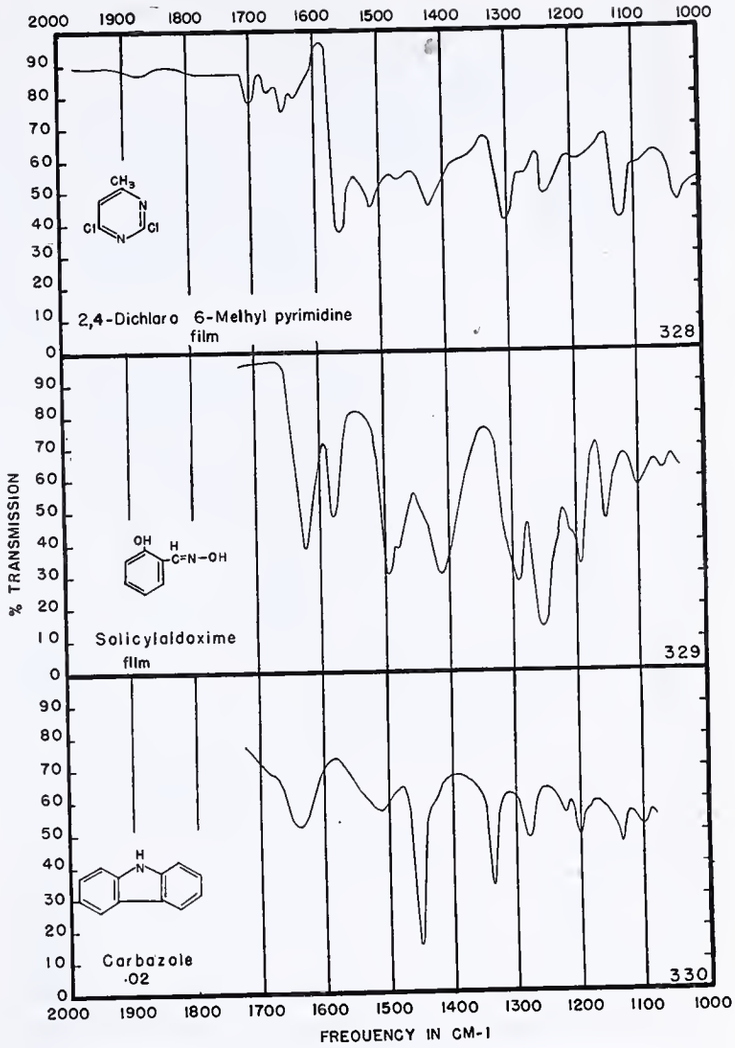

% TRANSMISSION
2,6-Lutidine
.02
313
2,4,6 Collidine
314
Pyridazine
045
315
FREQUENCY IN CM-1

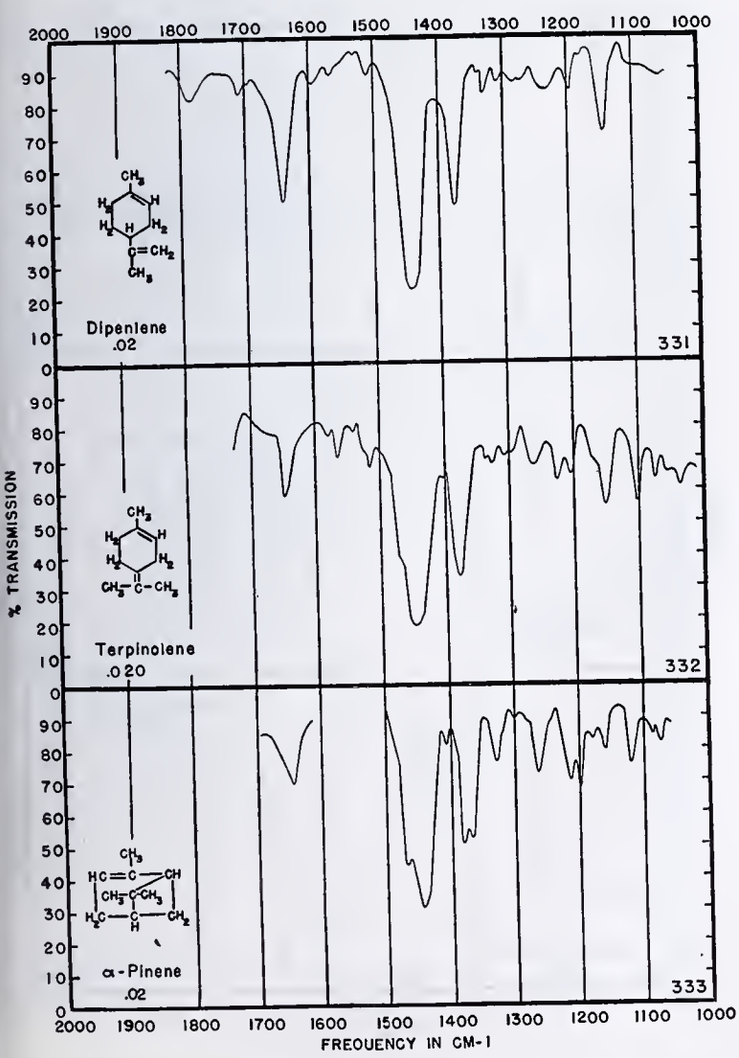

% TRANSMISSION
Pyrimidine
.045
Pyrozine
.02
2-Amino 4-Chloro pyrimidine
film
318
FREQUENCY IN CM-1

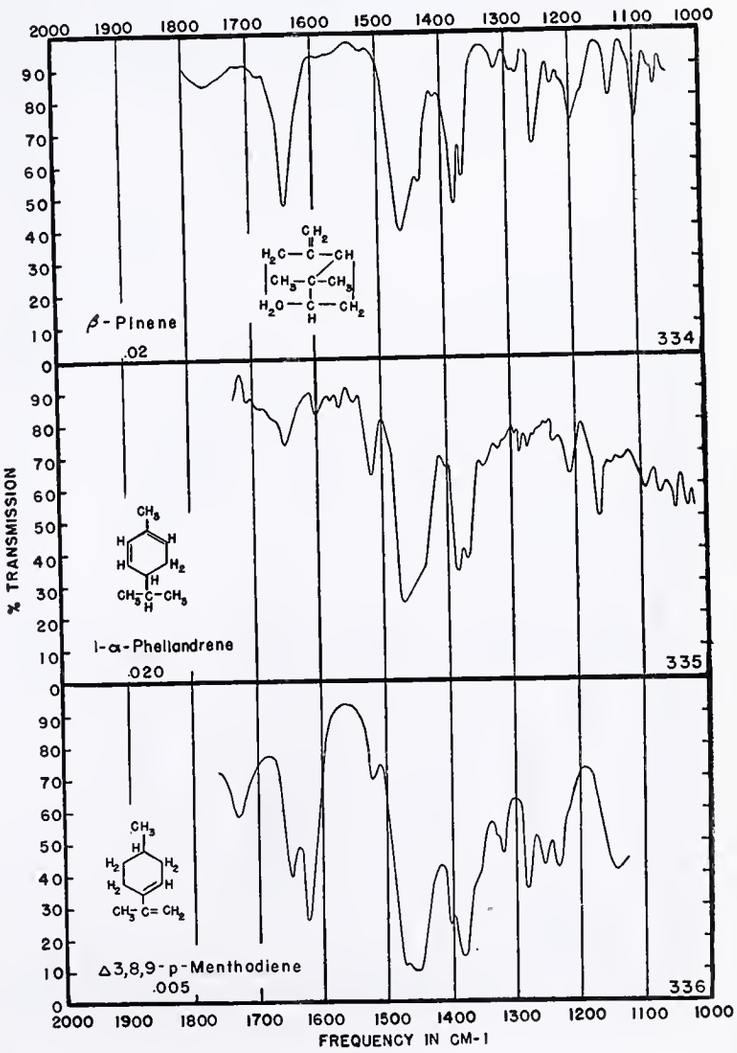

% TRANSMISSION
Pyrrole
.02
319
Diamyl melamine
320
Azobenzene
.02
321
FREQUENCY IN CM-1

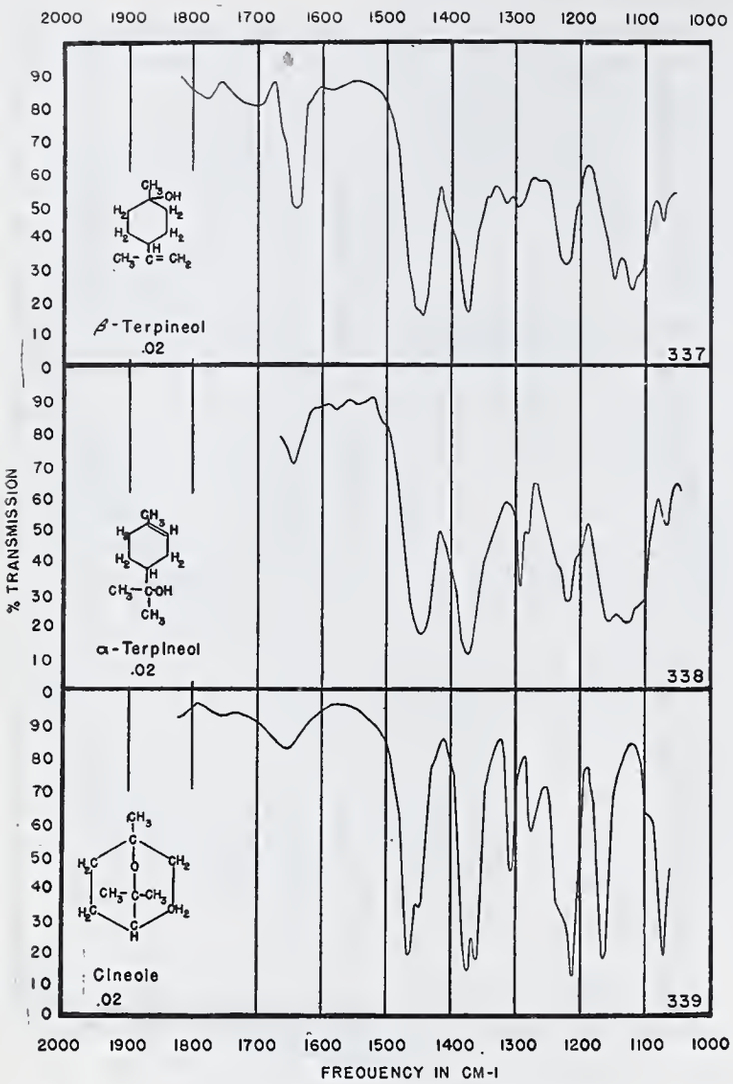

% TRANSMISSION
Quinoline
045
Adenine Sulfate in Nujol
.045
Dithio diethyl amine
.02
FREQUENCY IN CM-1

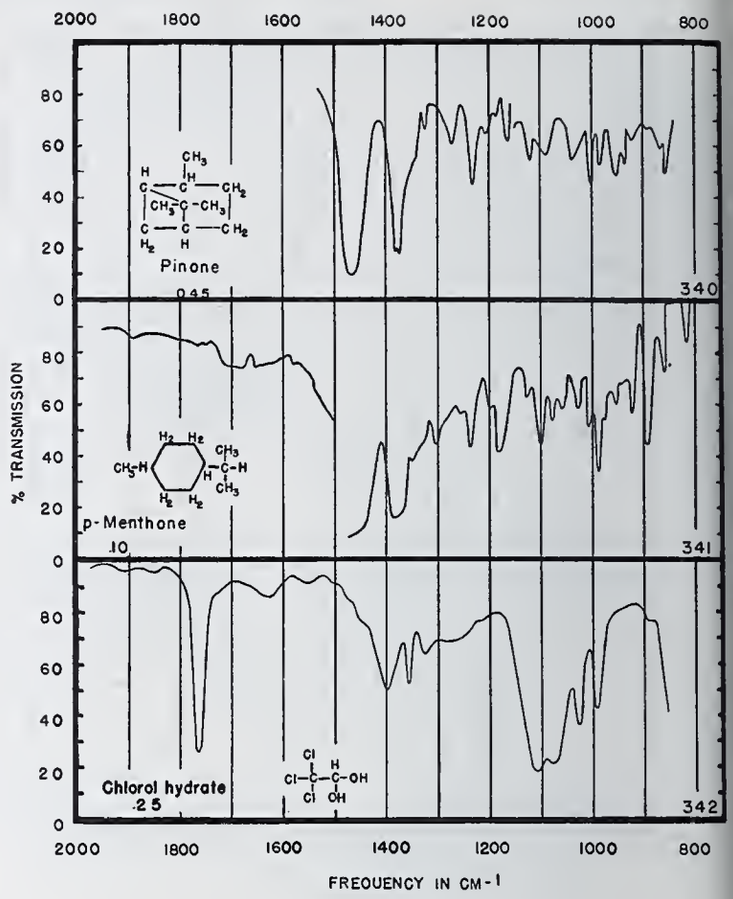

Riboflavin in Nujol
.045
325
Dehydro thio-p-toluidine in Nujol
.045
326
Dehydro-Abietic acid in CCl_4
.10
327
% TRANSMISSION
FREQUENCY IN CM-1

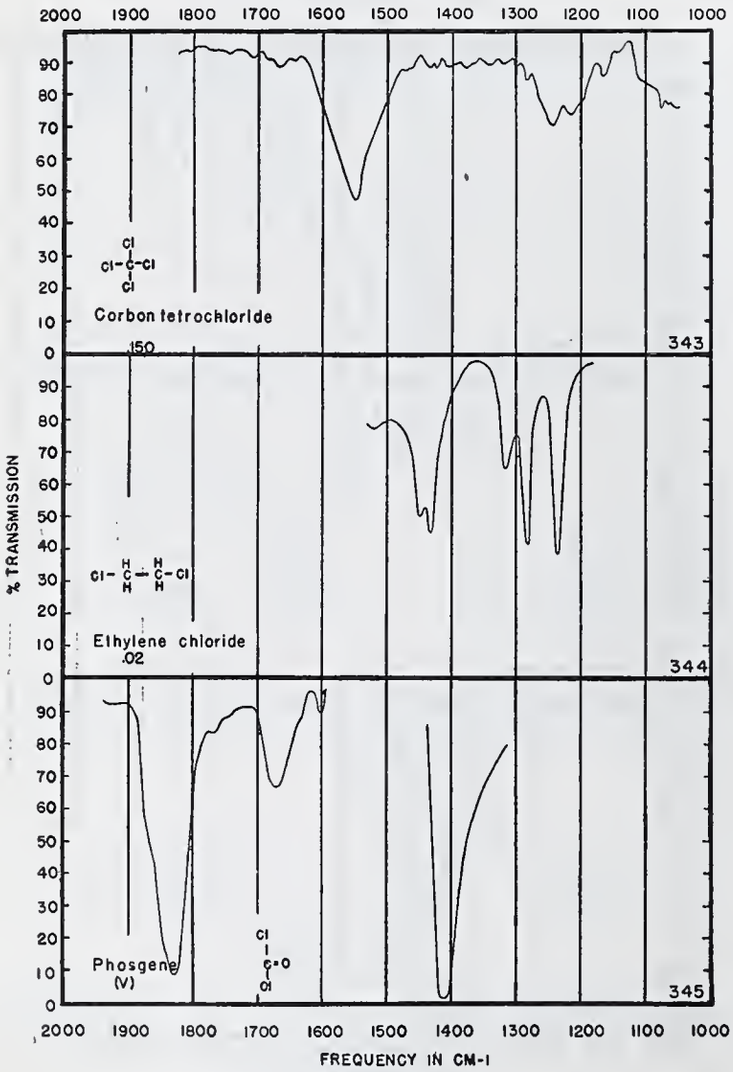

2,4-Dichloro 6-Methyl pyrimidine
film
328
Salicylaldoxime
film
329
Carbazole
.02
330
% TRANSMISSION
FREQUENCY IN CM-1

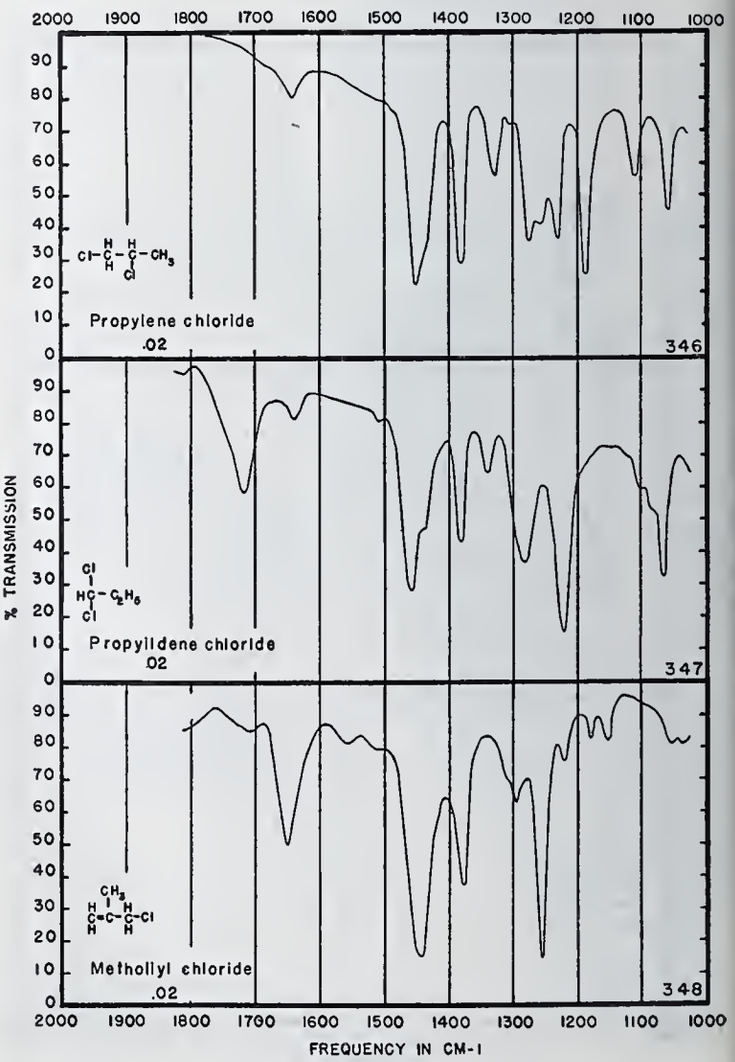

Dipentene
.02
331
Terpinolene
.020
332
α-Pinene
.02
333
% TRANSMISSION
FREQUENCY IN CM-1

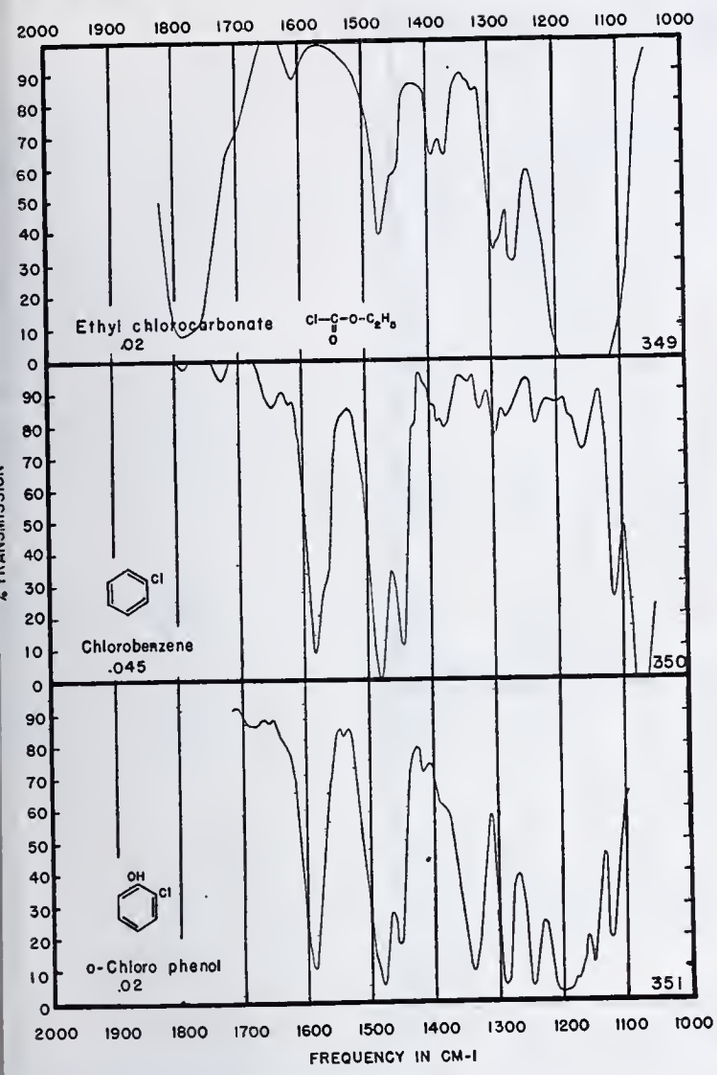

β-Pinene
.02
334
l-α-Phellandrene
.020
335
Δ3,8,9-p-Menthodiene
.005
336
% TRANSMISSION
FREQUENCY IN CM-1

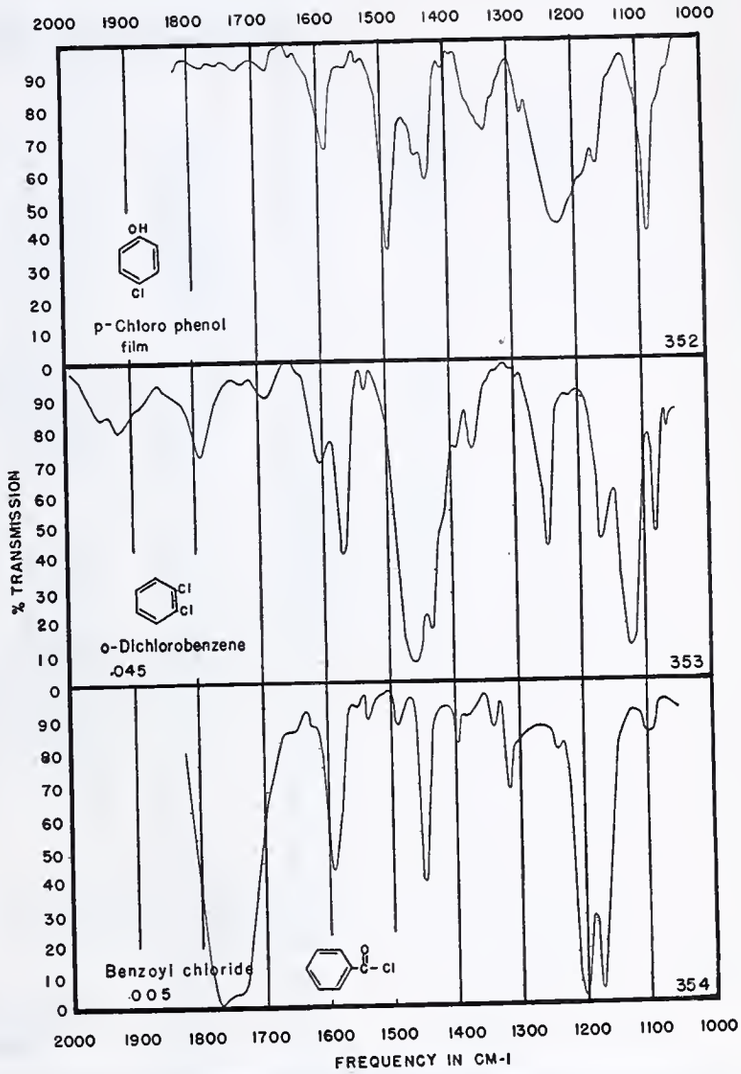

β-Terpineol
.02
337
α-Terpineol
.02
338
Cineole
.02
339
% TRANSMISSION
FREQUENCY IN CM-1

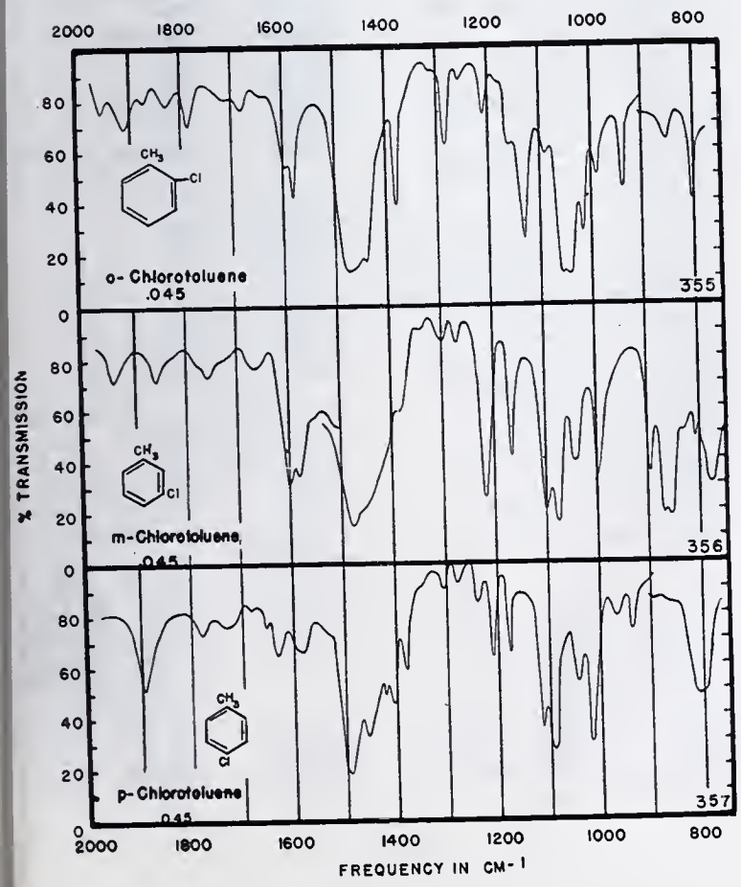

Pinone
.045
340
p-Menthone
.10
341
Chlorol hydrate
.25
342
% TRANSMISSION
FREQUENCY IN CM-1

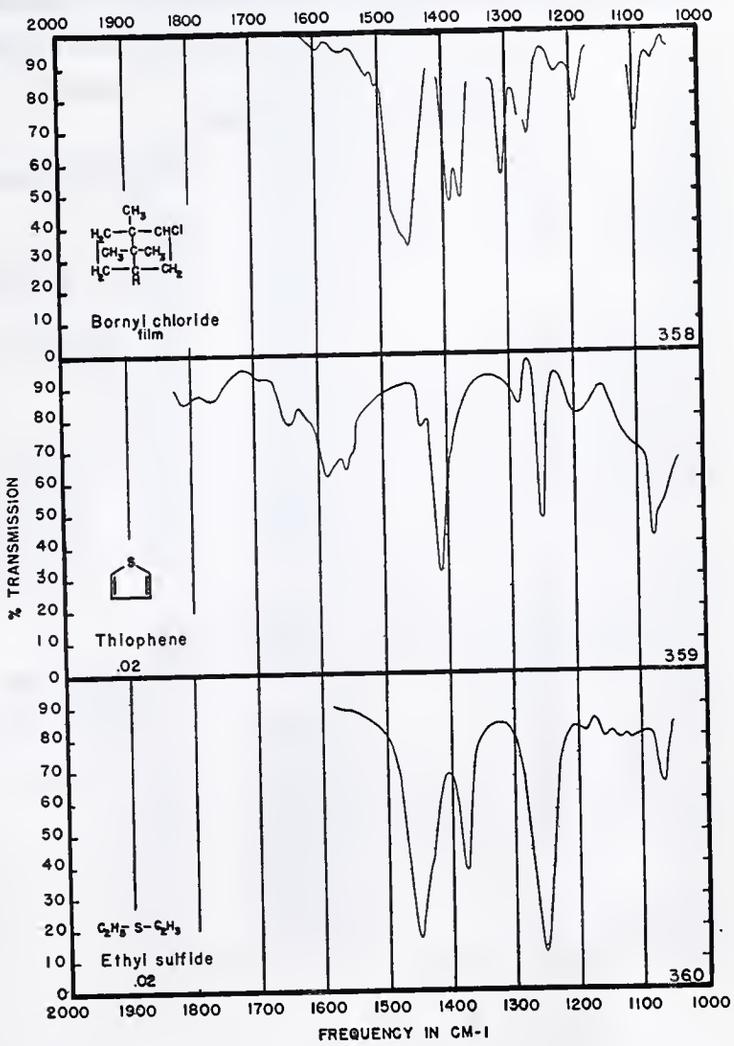

Carbon tetrachloride
.150
343
Ethylene chloride
.02
344
Phosgene
(V)
345
% TRANSMISSION
FREQUENCY IN CM-1

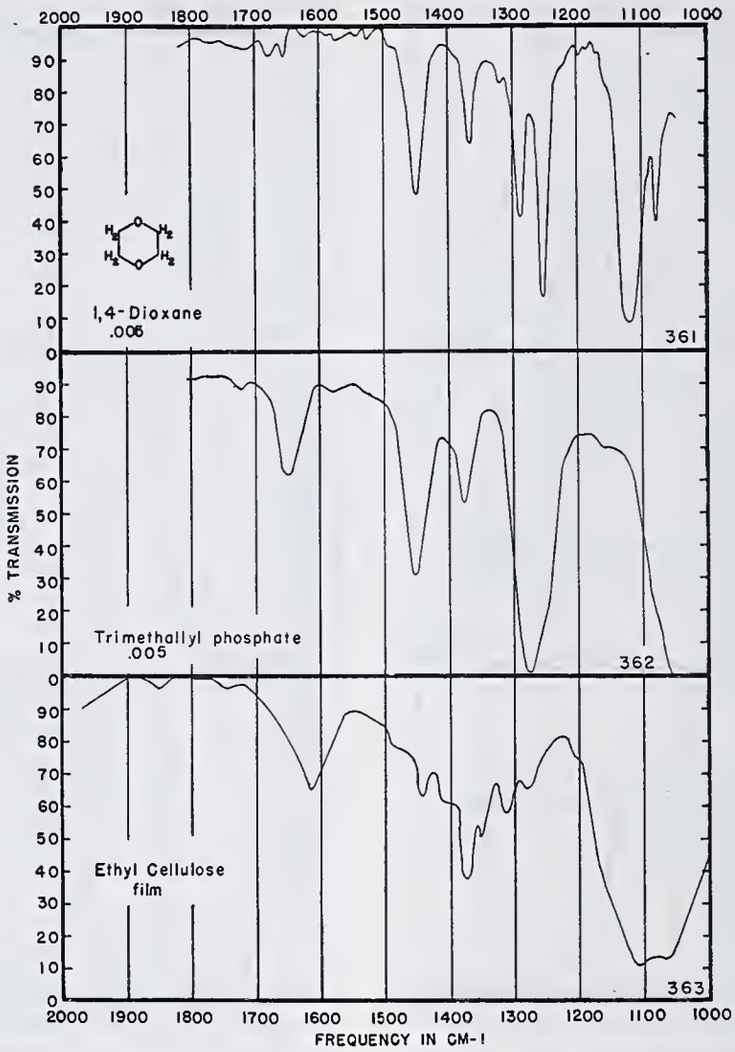

Propylene chloride
.02
346
Propylidene chloride
.02
347
Methallyl chloride
.02
348
% TRANSMISSION
FREQUENCY IN CM-1


Ethyl chlorocarbonate
.02
349
Chlorobenzene
.045
350
o-Chloro phenol
.02
351
FREQUENCY IN CM-1


% TRANSMISSION
p-Chloro phenol
film
352
o-Dichlorobenzene
.045
353
Benzoyl chloride
.005
354
FREQUENCY IN CM-1


% TRANSMISSION
o-Chlorotoluene
.045
355
m-Chlorotoluene
.045
356
p-Chlorotoluene
.045
357
FREQUENCY IN CM-1


% TRANSMISSION
Bornyl chloride
film
358
Thiophene
.02
359
Ethyl sulfide
.02
360
FREQUENCY IN CM-1



EMPIRICAL FORMULA INDEX TO REFERENCE CURVES

	Curve No.
C_1	
CCl_2O	345
CCl_4	343
CH_2N_2	270
CH_2O	208
CH_4N_2O	263
CH_4O	79
C_2	
$C_2H_3Cl_3O_2$	342
C_2H_3N	277
$C_2H_4Cl_2$	344
$C_2H_4N_4$	271
$C_2H_5NO_2$	292
$C_2H_5NO_3$	293
C_2H_6O	80
$C_2H_6O_2$	91
C_3	
C_3H_3N	279
C_3H_5ClO	153
$C_3H_5ClO_2$	349
$C_3H_6Cl_2$	346, 347
$C_3H_6N_2$	272
C_3H_6O	86, 217
$C_3H_6O_2$	121, 161
$C_3H_7ClN_2O$	275
$C_3H_7NO_2$	290
$C_3H_7NO_3$	294
C_3H_8O	81, 82
$C_3H_8O_2$	96
C_4	
$C_4H_2O_3$	156
$C_4H_4ClN_3$	318
$C_4H_4N_2$	315, 316, 317
C_4 (Contd.)	
C_4H_4S	359
C_4H_5N	278, 280, 286, 287, 319
C_4H_8	26
C_4H_8O	209
$C_4H_8O_2$	166, 168
$C_4H_8O_3$	155
C_4H_7Cl	348
C_4H_8	24, 25, 28
C_4H_8O	87, 88
$C_4H_8O_2$	162, 361
$C_4H_8O_3$	227
C_4H_{10}	22, 23
$C_4H_{10}O$	83, 84, 94, 124
$C_4H_{10}O_2$	92
$C_4H_{10}O_3$	133
$C_4H_{10}S$	360
$C_4H_{11}N$	235
C_5	
$C_5H_4Cl_2N_2$	328
$C_5H_4O_2$	210
C_5H_7N	281, 282, 283, 284, 285, 288
$C_5H_7N_5O_4S$	323
C_5H_8	27, 29
C_5H_8O	218
$C_5H_8O_2$	167, 169, 172
$C_5H_{10}N_2$	273
$C_5H_{10}O$	129
$C_5H_{10}O_2$	219
$C_5H_{10}O_3$	220
C_5H_{12}	1
$C_5H_{12}O$	85
$C_5H_{13}N_3O_2$	240
C_6	
$C_6H_4Cl_2$	353
$C_6H_4N_2O_4$	295, 296, 297
C_6H_5Cl	350
C_6H_5ClO	351, 352
$C_6H_5NO_2$	291
$C_6H_5NO_3$	298, 299, 300
C_6H_6	44
C_6H_6ClN	247, 248, 249
$C_6H_6N_2O_2$	301, 302, 303
C_6H_6O	100
$C_6H_6O_2$	119, 120
C_6H_7N	241, 308, 309, 310
$C_6H_7NO_3S$	253, 254, 255
$C_6H_8N_2O_2S$	256, 257, 258, 259
$C_6H_8O_4$	179, 184
C_6H_{10}	42
$C_6H_{10}O$	151, 221, 224
$C_6H_{10}O_2$	173, 222
$C_6H_{10}O_3$	178
C_6H_{12}	37, 38
$C_6H_{12}O$	97
C_6H_{14}	2
$C_6H_{14}O$	125, 130
$C_6H_{16}N$	237
C_7	
C_7H_5ClO	354
C_7H_5N	289
C_7H_5NO	223
$C_7H_5N_3O_5$	307
C_7H_5O	211
$C_7H_5O_2$	212
$C_7H_5O_3$	229, 230
C_7H_7BrO	138
C_7 (Contd.)	
C_7H_7Cl	355, 356, 357
C_7H_7NO	264
$C_7H_7NO_2$	265, 329
C_7H_8	71
$C_7H_8N_2O$	268
C_7H_8O	89, 134
$C_7H_8O_2$	122
C_7H_9N	242, 244, 245, 246, 311, 312, 313
$C_7H_{10}O_2$	174
$C_7H_{10}O_4$	187
$C_7H_{12}O_2$	171
C_7H_{14}	39
$C_7H_{14}O$	98
$C_7H_{14}O_3$	164
C_7H_{15}	3, 9
$C_7H_{17}N$	236
C_8	
$C_8H_4O_3$	159
C_8H_8	72
$C_8H_8N_2$	276
C_8H_8O	213, 225
$C_8H_8O_2$	231, 232, 233
C_8H_9NO	266
C_8H_{10}	45, 52, 53, 54
$C_8H_{10}N_2O$	267
$C_8H_{10}O$	101, 102, 103, 112, 113, 114, 115, 116, 135, 136, 137, 143
$C_8H_{10}O_2$	123, 142
$C_8H_{10}O_3$	157
$C_8H_{11}N$	243, 250, 251, 314
C_8H_{12}	30

EMPIRICAL FORMULA INDEX TO REFERENCE CURVES (Contd.)

	Curve No.
C_8 (Contd.)	
$C_8H_{12}O_4$	180, 185
$C_8H_{14}O_4$	163
C_8H_{16}	31, 40
C_8H_{18}	4, 10, 11, 13
$C_8H_{18}O$	126
$C_8H_{19}N$	238
C_9	
C_9H_7N	322
C_9H_8	77
C_9H_{10}	73
C_9H_{12}	46, 47, 56, 57, 58
$C_9H_{12}O$	144, 145, 146, 148
C_9H_{18}	41
C_9H_{20}	5, 12, 14, 16, 17, 18
C_{10}	
$C_{10}H_8$	67
$C_{10}H_8O$	118
$C_{10}H_9N$	260, 261
$C_{10}H_{10}O_4$	190
$C_{10}H_{12}$	74, 75, 76
$C_{10}H_{12}N_2O_5$	304, 305
$C_{10}H_{12}O$	214
$C_{10}H_{14}$	48, 49, 50
$C_{10}H_{14}O$	104, 117, 149
C_{10} (Contd.)	
$C_{10}H_{16}$	70, 331, 332, 333, 334, 335, 336
$C_{10}H_{16}O$	216
$C_{10}H_{17}Cl$	358
$C_{10}H_{18}$	340
$C_{10}H_{18}O$	337, 338, 339
$C_{10}H_{20}$	341
$C_{10}H_{20}O$	99
$C_{10}H_{22}$	6, 15, 19
$C_{10}H_{22}O$	127, 128
$C_{10}H_{22}O_2$	93
$C_{10}H_{23}N$	239
C_{11}	
$C_{11}H_{16}$	51
$C_{11}H_{16}O$	105, 131, 150
$C_{11}H_{22}O_2$	228
$C_{11}H_{24}$	7
C_{12}	
$C_{12}H_8O$	152
$C_{12}H_9N$	330
$C_{12}H_{10}$	59
$C_{12}H_{10}N_2$	321
$C_{12}H_{10}O$	108, 109, 154
$C_{12}H_{11}N$	262
$C_{12}H_{12}N_2$	252
C_{12} (Contd.)	
$C_{12}H_{14}N_2O_5$	306
$C_{12}H_{18}O$	139
$C_{12}H_{18}O_4$	188
$C_{12}H_{20}O_4$	181, 186
$C_{12}H_{21}PO_3$	362
$C_{12}H_{22}O_3$	158
$C_{12}H_{24}$	32
$C_{12}H_{25}O_3$	363
$C_{12}H_{26}$	8, 20
C_{13}	
$C_{13}H_{10}O$	110, 111, 226
$C_{13}H_{12}O$	140, 141
$C_{13}H_{22}N_2$	274
$C_{13}H_{26}N_6$	320
$C_{13}H_{26}O_2$	175
C_{14}	
$C_{14}H_{10}$	68
$C_{14}H_{10}O_4$	215
$C_{14}H_{12}$	61
$C_{14}H_{14}$	60
$C_{14}H_{14}O$	132, 147
$C_{14}H_{14}O_4$	191
C_{15}	
$C_{15}H_{30}$	35
$C_{15}H_{32}$	21
C_{16}	
$C_{16}H_{22}O$	106, 107
$C_{16}H_{22}O_4$	192
$C_{16}H_{26}O_4$	189
$C_{16}H_{32}$	33
$C_{16}H_{34}$	43
C_{17}	
$C_{17}H_{20}N_2O$	269
C_{18}	
$C_{18}H_{14}$	63, 64, 65
$C_{18}H_{18}$	69
$C_{18}H_{26}O_4$	193
C_{19}	
$C_{19}H_{16}$	62
$C_{19}H_{16}O$	90
$C_{19}H_{36}O_2$	176
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$C_{24}H_{18}$	66

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BOOK REVIEWS

A. S. T. M. Methods of Chemical Analysis of Metals

The 1943 volume of A. S. T. M. Methods of Chemical Analysis of Metals contains tentative recommended practices for reagents and apparatus, two sampling methods, and seventeen standard and tentative methods of chemical analysis, comprising four methods for analyzing ferrous metals, ten methods for nonferrous metals and alloys, and three methods of quantitative spectrochemical analysis of nonferrous metals. Published as information only are methods for the chemical analysis of special brasses and bronzes, and of white metal bearing alloys; also a procedure for aluminum in solder metal, and methods of sampling wrought metals and alloys for determination of chemical composition. The book also includes a list of standard samples of metals issued by the National Bureau of Standards and a good index.

Work leading to the issuance of the book has been under way for many months, particularly in Committee E-3 on Chemical Analysis of Metals headed by G. E. F. Lundell of the National Bureau of Standards. Committee E-2 on Spectrographic Analysis formulated the methods of spectrochemical analysis.

The book, 323 pages, is available at $3 per copy from the American Society for Testing Materials, 260 South Broad St., Philadelphia 2, Penna. Individual test methods are available in separate pamphlet form.

Spectrographic Analysis

Two tentative methods developed by Committee E-2 of the American Society for Testing Materials cover tentative spectrochemical methods for the determination of impurities in tin alloys (E51-43T) and in lead alloys (E49-43T).

The method involving lead alloys gives a procedure for the determination of 0.001 to 0.30 per cent of antimony, arsenic, barium, bismuth, copper, iron, magnesium, nickel, silver, strontium, tin, and zinc in lead, lead-calcium, and lead-antimony alloys, with an average precision of ±10 per cent of the amount of the element sought. It is also applicable to other alloys of high lead content except those containing a large proportion of tin.

The method for tin alloys covers the determination of 0.001 to 1.0 per cent of antimony, and 0.001 to 0.3 per cent of aluminum, arsenic, bismuth, copper, iron, lead, nickel, and zinc in solders, bronzes, tin base die-cast alloys, and other alloys high in tin.

An additional procedure making use of photometric measurement has been included in the tentative method of quantitative spectrochemical analysis of zinc alloy die castings for minor constituents and impurities (E27-40T).

The revised methods of test for quantitative spectrochemical analysis of zinc for lead, iron, and cadmium (E26-35T) may be applied to any grade of zinc, providing the lead, iron, and cadmium contents are less than 0.1 per cent.

Semimicro Qualitative Analysis. *James T. Dobbins.* x + 423 pages. John Wiley & Sons, Inc., New York, N. Y., 1943. Price, $3.00.

A textbook containing a set of experiments illustrating the fundamental principles; a complete systematic scheme of separation of the anions; and methods reducing time required for a complete analysis.

Semimicro Qualitative Analysis. *John F. Flagg* and *Willard R. Lin*, viii + 140 pages. D. Van Nostrand Co., Inc., New York, N. Y 1943. Price, $1.50.

A course in applied chemical equilibrium. Includes chapters o: basic theory and notes adjoining the laboratory procedures, in whicl the application of the theory is pointed out.

Delivery of Laboratory Equipment

Limitation Order L-144, governing the delivery of laboratory equipment, was amended October 9, 1943, in order to simplify anc clarify the provisions of the order and reduce the number of applications to be filed by users of laboratory equipment.

Restrictions are entirely eliminated on the delivery of any item costing less than $50, a quantity of the same item costing $50 or more a miscellaneous order aggregating $200 or more, accessories anc attachments when sold separately, or parts or materials to be used for repair and maintenance of existing instruments.

Form WPB-1414 is required for instruments included in List A costing $50 or more.

List A. Analytical balances (sensitivity 0.2 mg. or more sensitive), calorimeters, centrifuges, hydrogen-ion meters (electrometric), metalloscopes, microscopes (except Brinell and tool makers), microtomes, potentiometers, Wheatstone bridges, and resistance boxes, refractometers, spectrographs, spectroscopes, spectrometers, and spectrophotometers, and vacuum pumps (1 micro or higher vacuum).

Laboratories' Rating for Reagent Chemicals Raised

Preference ratings on deliveries of reagent chemicals for laboratory use were reassigned September 28 by the War Production Board through issuance of Preference Rating Order P-135 as amended. Preference rating AA-1 is assigned to deliveries of any reagent chemical to any laboratory to which a serial number has been assigned under Preference Rating Order P-43, governing laboratory equipment, and to any laboratory owned and operated by the Army or Navy. Preference Rating AA-2 is assigned to deliveries of any reagent chemical to any laboratory lacking a serial number under Order P-43 or to a distributor or producer of reagent chemicals. Order P-135 formerly assigned a blanket rating of AA-2X to reagent chemicals for laboratories.

The amended order defines "laboratory" as meaning "any person engaged in the business of carrying on scientific or technological investigation, testing, development or experimentation, to the extent that he is so engaged. The term includes research laboratories, production control laboratories, clinical laboratories, and instructional laboratories. It does not include any person to the extent that he is engaged in the manufacture of products for commercial sale, even though the place in which the products are manufactured may be called a laboratory."

Supplementary Order P-135-a also was amended to exempt from certification the delivery to or acceptance of delivery by a laboratory of not more than 10 per cent of the quantity of reagent chemicals in any period which are exempt from specific authorization under the small order clause.

INDUSTRIAL AND ENGINEERING CHEMISTRY

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Polymer Distribution of Varnish Resins

H. E. ADAMS AND P. O. POWERS, Armstrong Cork Company, Lancaster, Penna.

An approximate estimate of the distribution of polymers in varnish resins may be made by precipitating the resin from dilute solution with measured amounts of a nonsolvent, while determining the decrease in light transmission through the medium. This titration is reasonably rapid and can be carried out in 20 minutes. This method has been used with hydrocarbon resins, rosin-modified phenolic and maleic resins, and pure phenolic resins using toluene as the solvent and methanol or hexane as the nonsolvent. The titration curves obtained in this manner may be used to compare resins of the same type. To estimate polymer distribution from these curves, the concentration of nonsolvent at which carefully separated fractions of a resin are precipitated must be determined.

THE distribution of polymers in commercial resins used in the manufacture of paints and varnishes has never been reported. Since these resins are considerably lower in molecular weight than the resins used as plastics whose polymer distribution has in many cases been established, the determination might be expected to be rather simpler. However, the polymers in commercial resins are often built up from several different types of molecules. This results in a greater complexity in the types of polymers formed.

Determination of the cloud point (*2*) of varnish resins in many instances gives a useful index of the solubility and hence the degree of polymerization of a resin. It has been found in some cases, however, that two resins of the same type and of the same cloud point are not identical in all their properties. This difference is probably due to a difference in the distribution of the polymers in the two resins. It was desired to develop a method that would quickly establish such differences.

Polymer distribution has often been effected by fractional precipitation of the resin from solution. The fractions are separated and their properties determined. This method is time-consuming and while it has been used in these laboratories, it is much too slow for routine examination of samples of resins.

Nephelometric methods have been used widely to measure the amount of a precipitate. McNally (*1*) has described a method for measuring the transmission of light through a solution of a synthetic resin during precipitation by a nonsolvent. A photronic cell was used to measure the transmission, which varied inversely with the amount of resin precipitated.

Schulz (*3*) has shown that the amount of nonsolvent required to precipitate a polymer is a measure of its degree of polymerization. Thus the distribution of polymers in a resin may be estimated if the transmission during precipitation and the concentration of nonsolvent required to precipitate a polymer are known. Even where the polymer-nonsolvent-concentration relationship has not been established, the results of precipitation are valuable in comparing resins of the same class.

Apparatus and Method

The titration was conducted in a square pint jar which had been painted black except for two windows on opposite sides about 2.5 × 7.5 cm. (1 × 3 inches). Light entered through one window from an automobile head lamp with a parabolic reflector, using a 6-volt storage battery as a source of current. The reflector was covered except for a small window, placed in front of the titration jar (Figure 1). The photronic cell (Weston No. 594) was placed in front of the other window. The circuit used to measure the cell voltage is shown in Figure 2.

A 1.5-volt dry cell was used as source of voltage. The poten-

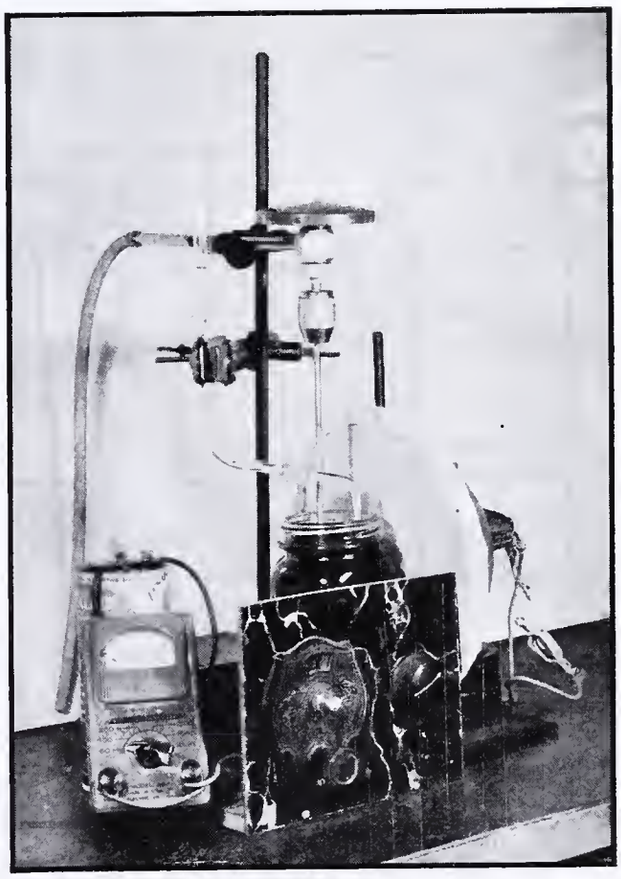


FIGURE 1. TITRATION APPARATUS

tiometer was wire-wound and had a spread of about 180°. A dial reading 0 to 100 was used to measure the position of the null point. Resistances R_1 to R_5 were carbon resistors. The values are those actually used, but are not critical. These resistors make it possible to titrate solutions of dark-colored resins, since changes in transmission at low intensities can be measured if a high resistance is used.

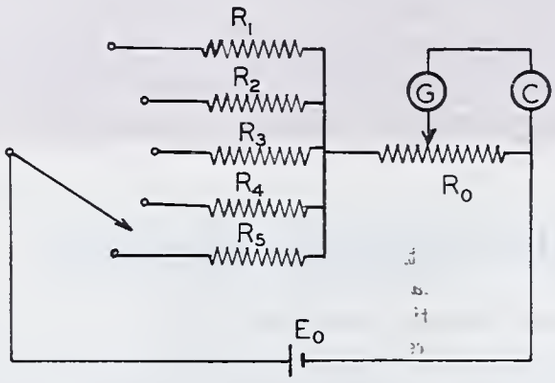


FIGURE 2. PHOTRONIC CELL CIRCUIT

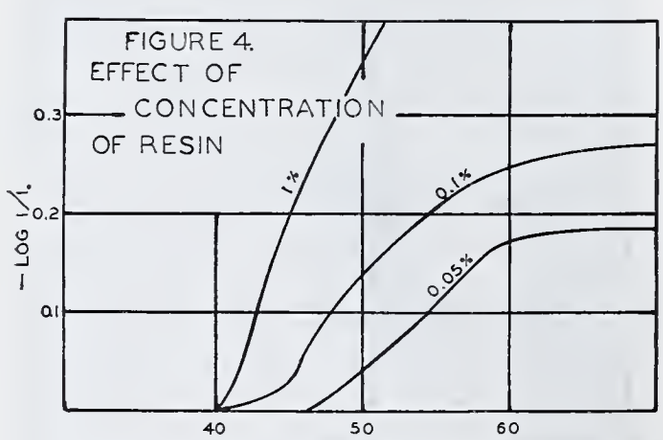
R_0.	1092 ohms	E_0.	1.5 volts
R_1.	957 ohms	C.	Photronic cell
R_2.	5108 ohms	G.	Microammeter
R_3.	9489 ohms		
R_4.	27,500 ohms		
R_5.	97,000 ohms		

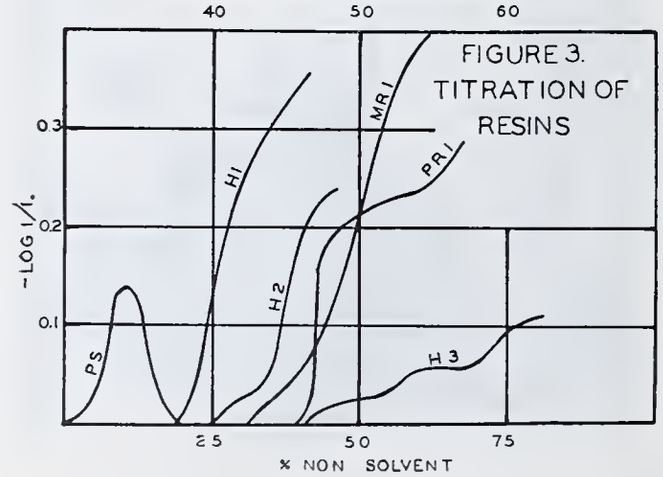
The solution of the resin and the nonsolvent were brought to 25° C. To conduct a titration, 80 cc. of the solution of the resin, usually in toluene at 0.05 per cent solids, were added to the jar and the nonsolvent was added with stirring by an air-driven agitator. The rate of stirring must be sufficiently great to ensure complete mixing, but must not cause the introduction of air bubbles into the solution. A nonsolvent, usually methanol, was added until it was apparent that precipitation was about to occur. The microammeter was balanced, using the switch setting giving the largest reading on the potentiometer scale. Small amounts of methanol were added and the circuit was again balanced. The reading just before precipitation started was taken as a measure of the initial transmission, I_0. Small amounts of precipitant were added and the transmission, I, was determined after each addition. Decrease in transmission occurs rapidly at first and the titration is complete when further addition of precipitant does not further decrease the transmission.

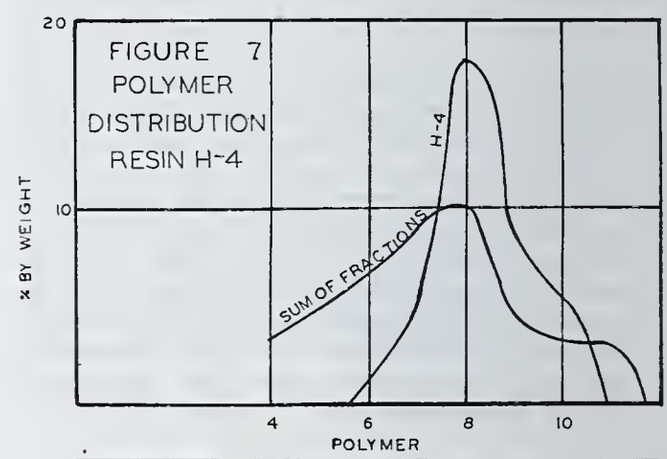
It might be expected that transmission would increase by dilution, but generally such increase was not observed. The apparatus and method were checked by precipitating inorganic salts and it was found the extinction, $-\log I/I_0$, measured the amount of the precipitate at low concentration.

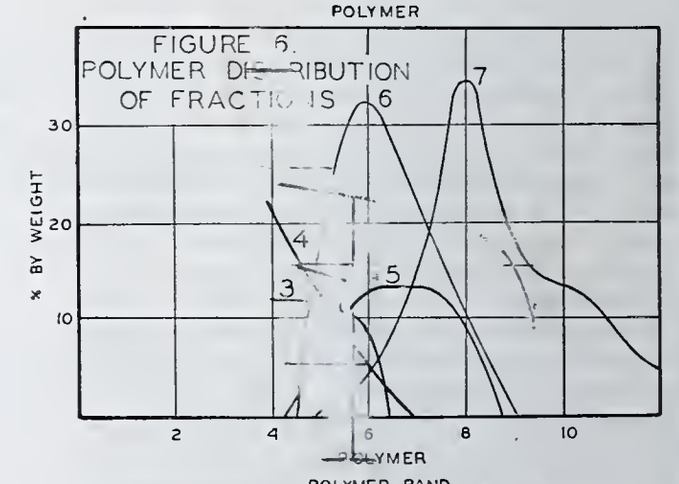
Titration of Varnish Resins

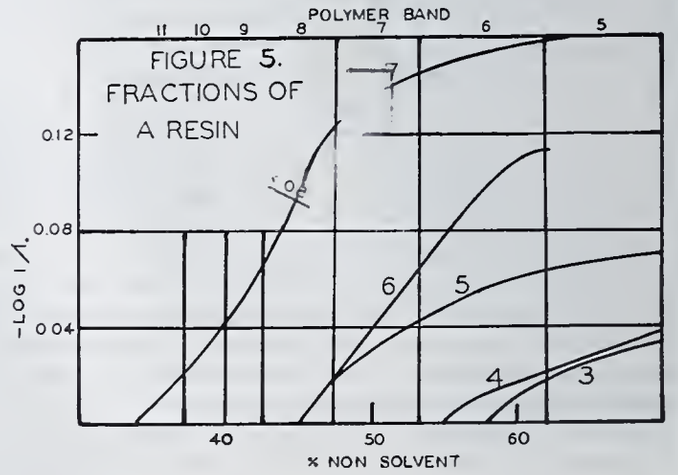
To establish the range of precipitation and conditions for titration, a variety of varnish resins was dissolved in toluene (0.5 per cent solution) and precipitated by the addition of methanol. Figure 3 shows that an estimate of polymer distribution is possible for several types of varnish resins. H1, H2, and H3 are coumarone-indene resins of successively decreasing molecular weight. A maleic rosin resin (MR1) and a rosin-modified phenolic (PR1) resin were also titrated, and the results are shown in Figure 3. These resins are typical of the less soluble varnish resins. The











results show the wide range of composition of commercial resins. Polystyrene (PS) is also included; it is much higher in molecular weight than the other resins. The decrease in transmission is due to the resin settling out of solution; this can be overcome by using a more dilute solution.

Titrations are probably more significant if run at lower concentration. Figure 4 shows the results of titrating the same resin at three different concentrations. It will be noted that higher concentration of nonsolvent may be required to start precipitation at low concentration. Estimates of molecular size from precipitability determinations may therefore depend on concentration of the resin, which should be constant for such determinations.

Polymer Distribution

The titration curves are believed to be a sufficient basis for comparison of commercial varnish resins. However, to correlate a titration with polymer distribution, a coumarone-indene resin was fractionated by partial solution, resulting in seven fractions, and the molecular weight of the fractions was determined by depression of the melting point of pure benzene.

The titration curves of these fractions are shown in Figure 5. The first two more soluble fractions did not precipitate on titration. The other fractions gave varying amounts of precipitate, only the last fraction being entirely precipitated, showing presence of lower polymers in these fractions.

Since the results of the titration and molecular weight determinations indicated that the decamer was precipitated at 37 per cent nonsolvent and the tetramer at 73 per cent nonsolvent, the Schulz (*3*) equation

$$\gamma^* = A + B/X$$

where

γ^* = concentration of nonsolvent
X = degree of polymerization

and

A and B = constants

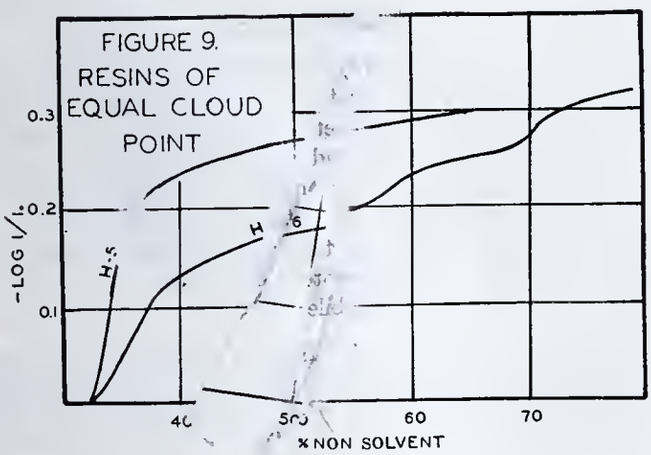
was used to estimate the range in which each polymer is thrown down. From the above values

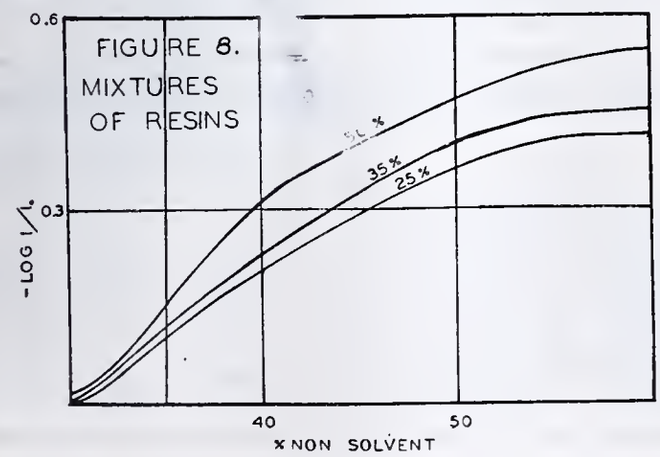
$$\gamma^* = 0.13 + \frac{2.42}{X}$$

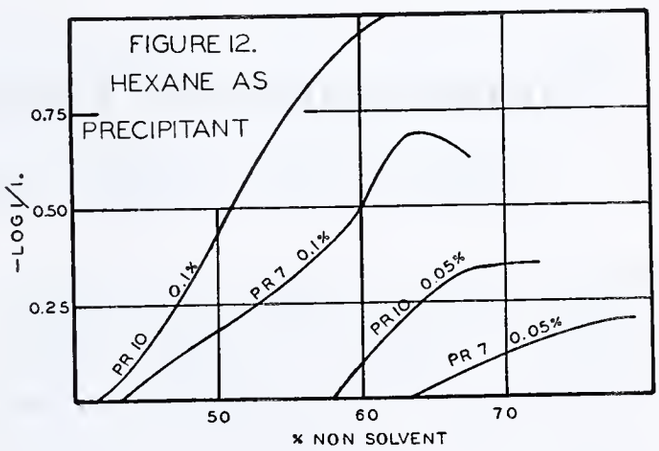
The range for each polymer by this relationship is shown in Figure 6. The amount in each fraction is estimated by the differential of $-\log I/I_0$ and the values for the fractions are summed in Figure 7. A similar estimate of the distribution of the resin was made from the titration of the original resin and, as might be expected, shows a somewhat narrower distribution. It is apparent that the lower polymers are not estimated by this method. The results also suggest a mutual solubility effect of various polymers, resulting in incomplete separation.

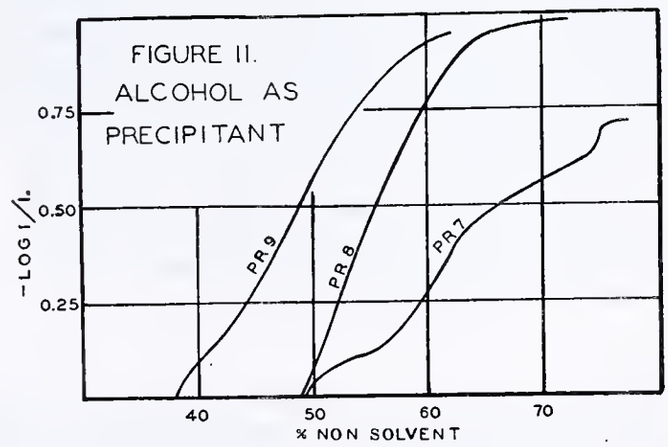
In many cases the high polymers determine the character of the resin, and an estimate of the range and amount of these polymers afford significant information on the composition of a resin.

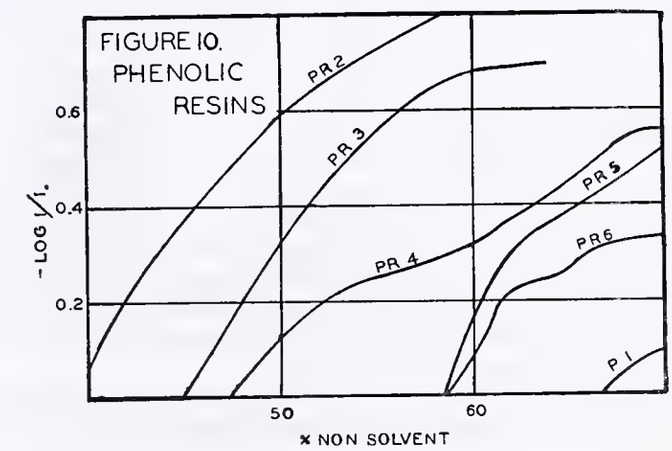
To determine if titration would measure small differences in the composition of a resin, mixtures of two hydrocarbon resins of somewhat similar properties were examined. It will be noted (Figure 8) that the curves indicate the presence of larger amounts of higher polymers as the content of the less soluble resin is in-











creased. Resins of equal cloud point may have somewhat different properties. The titration curve of two such resins, H5 and H6, is shown in Figure 9. Resin H5 has a higher content of large polymers.

Titration of Rosin-Modified Phenolic Resins

Titration curves were determined for samples of several commercial rosin-modified phenolic resins, PR2–6, and of a "pure" phenolic resin, P1 (Figure 10). The resins having the greatest amount of material precipitated, as measured by $-\log I/I_0$, at low nonsolvent concentrations are the more viscous, higher melting, and less soluble. The pure phenolic, P1, is one of the more soluble of its class.

No attempt has been made to estimate polymer distribution from these titrations, as data are not available on molecular weight and precipitability of fractions of these resins. However, it is known that ester gum is not precipitated under the conditions of titration. Thus the material precipitated is phenolic resin and condensates of the phenolic resin with rosin glyceride.

A series of rosin-modified phenolic resins, PR7, 8, 9, and 10, was titrated. These resins differ chiefly in the content of phenolic resin which increases in each successive sample. The precipitation with methanol is in agreement with this composition (Figure 11). However, the results with PR10 were unexpected, since its titration curve nearly coincided with that of PR7. It is believed that the higher content of polar groups introduced by the higher phenolic content is responsible for this result. Titrating PR7 and PR10 with hexane the resins are in the expected order. The results at two concentrations are shown in Figure 12.

Conclusions

A rapid and simple method for the estimation of polymer distribution by titration of a solution of a varnish resin with nonsolvent has been used with hydrocarbon and modified phenolic resins, and minor differences in resins can be quickly shown. The method outlined here is tentative and better conditions of determination are undoubtedly possible.

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(2) Powers, P. O., Ind. Eng. Chem., Anal. Ed., 14, 387 (1942).
(3) Schulz, G. V., *Z. Phys. Chem.*, A179, 321 (1937).

Presented before the Division of Paint, Varnish, and Plastics Chemistry at the 106th Meeting of the American Chemical Society, Pittsburgh, Penna.

Constituents of Carotene Extracts of Plants

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The crude carotene extracts of a number of materials as analyzed by adsorption in calcium hydroxide contained from 2.8 to 39.5 per cent of impurity A, which consists of several pigments, 26.4 to 95.4 per cent of beta-carotene, 0 to 18.1 per cent of a neo-beta-carotene, 0 to 26.7 per cent of a new pigment termed provisionally carotenoid X, and in a few cases alpha-carotene and neo-alpha-carotene. Biological assays show that carotenoid X does not possess vitamin A activity and that the neo-beta-carotene found has approximately one half the potency of beta-carotene. The carotene solutions prepared by several widely used methods for carotene were found to contain appreciable amounts of impurities, especially carotenoid X. All the methods heretofore proposed give only approximately correct results for carotene.

THE exact determination of carotenes in foods and feeds is of great practical and scientific importance because of their vitamin A potency. Beadle and Zscheile (*2*) have recently shown that a neo-beta-carotene is present in carotene extracts of fresh spinach and other fresh vegetables; the relative quantities of beta- and the neo-beta-carotene were calculated from light-absorption data obtained with a photoelectric spectrophotometer. Chromatographic analyses have shown that magnesium oxide will separate cryptoxanthol, neo-cryptoxanthol, beta-carotene, K-carotene, alpha-carotene, and unidentified pigments (*3*) which are not biologically active. According to Gillam, El Ridi, and Kon (*6*) and Beadle and Zscheile (*2*), magnesium oxide does not separate neo-beta-carotene from beta-carotene. Zechmeister and Tuzson (*16*) report that calcium hydroxide separates them.

Preliminary work in this laboratory showed that calcium hydroxide used in chromatographic analyses separated not only a neo-beta-carotene from beta-carotene, but also a carotenoid which has not previously been reported to occur naturally, here termed carotenoid X. Further study of the constituents of crude carotene obtained by different methods and from various sources is thus necessary.

Experimental

Zechmeister and Tuzson (*16*) and Beadle and Zscheile (*2*) have pointed out that beta-carotene partially isomerizes into pseudo-alpha-carotene (a neo-beta-carotene) when it is warmed in various solvents. Polgár and Zechmeister (*13*) by various treatments produced about 12 isomers of beta-carotene. They found that after refluxing beta-carotene in petroleum ether the relative photometric ratios of the four pigments formed were neo-beta-carotene U 4, beta-carotene 86, neo-beta-carotene B 8, neo-beta-carotene E 1, and a labile isomer 1.

In the authors' laboratory solutions of crystalline commercial carotene which had been purified by solution in chloroform and precipitation with methyl alcohol (*5*) were subjected to the treatments shown in Table I and the pigments were then separated by

Table I. Effect of Treatment on Constituents of Purified Carotene

Treatment	Impurity %	Beta-Carotene %	Neo-Beta-Carotene %	Alpha-Carotene %
Dissolved in petroleum ether at room temperature	1.2	82.2	0.0	16.6
Boiled 30 minutes in hexane solution	0.6	72.9	9.0	17.5
Boiled 30 minutes in ethanol	0.6	65.9	18.8	14.7
Boiled 30 minutes in alcoholic KOH	0.3	59.8	17.1	22.8
Saponified 5 minutes in alcoholic KOH at room temperature	0.5	83.3	0.0	16.2
Boiled 30 minutes in methanol	1.0	71.7	10.4	16.9

Table II. Constituents of Crude Carotene

	Crude Carotene, A. O. A. C. Method	Crude Carotene, Cold Saponification	Loss in $Ca(OH)_2$ Column	Constituents: Impurity A	Carotenoid X	Beta-carotene	Neo-beta-carotene	Alpha-carotene
	P. p. m.	*P. p. m.*	%	%	%	%	%	%
Alfalfa, dehydrated	25.0	23.5	3.7	27.0	0.0	66.4	6.6	...
	200.0	172.5	4.8	9.4	12.6	65.9	10.1	2.0
	...	31.7	7.9	30.8	26.1	37.5	5.6	...
	...	105.0	10.5	14.9	20.2	46.8	18.1	...
	...	142.0	8.8	12.7	20.5	50.2	16.6	...
	...	102.5	2.8	29.7	26.7	26.4	17.2	...
	...	120.0	8.7	10.7	0.0	75.3	14.0	...
	...	188.3	0.4	5.8	23.1	53.3	17.8	...
Apricots, canned	12.8	11.4	3.1	30.8	5.9	56.1	7.2	...
Bermuda grass, fresh	56.4	54.0	7.4	6.4	8.4	80.0	5.2	...
Bur clover, fresh	...	80.0	12.5	3.7	0.0	89.1	7.2	...
Carrots, fresh	36.9	39.3	2.5	6.5	0.0	64.7	0.0	28.8
Carrots, dehydrated	...	648.0	7.4	5.7	0.0	63.3	2.3	28.7
	...	1048.0	6.4	2.8	1.2	66.4	3.3	26.3
Collards, fresh	38.8	42.0	6.5	5.7	8.0	79.0	7.3	...
Hegari silage	4.8	5.1	20.3	31.1	16.0	52.9	0.0	...
Johnson grass, fresh	144.8	138.0	11.7	6.7	12.4	73.1	6.5	1.3
Oat plants, fresh	52.0	53.6	0.4	6.3	9.4	78.0	6.3	...
Sumac sorghum silage	...	4.0	10.0	39.5	18.8	37.5	4.2	...
	10.8	10.0	9.0	32.5	14.5	45.6	7.4	...
Sweet potatoes, fresh	45.0	43.0	2.8	11.7	5.4	77.8	3.7	1.4
Swiss chard, fresh	59.8	59.8	1.5	3.6	0.0	83.5	11.2	1.7
Turnip greens, fresh	42.8	41.3	8.5	4.6	0.0	95.4	0.0	0.0

chromatographic analysis in a column of calcium hydroxide. The amounts of each pigment were determined in terms of carotene by means of a KWSZ photoelectric colorimeter (*9*).

The purified carotene and the purified carotene treated with alcoholic potash at room temperature did not contain neo-beta-carotene. When the carotene was boiled in a hexane solution for 30 minutes, the resulting pigments contained 9.0 per cent of a neo-beta-carotene, probably neo-beta-carotene B (*13*); when boiled in methanol, 10.4 per cent; when boiled with alcoholic potassium hydroxide, 17.1 per cent; and when boiled with ethanol, 18.8 per cent. This shows that methods which require heat cannot be used in extracting carotene from plants when neo-beta-carotene and beta-carotene is to be determined.

In order to ascertain the percentages of the constituents in the carotene extracts of various materials, and to avoid the formation of neo-beta-carotenes, a method of saponification at room temperature was used. Enough of the material to furnish 100 to 200 micrograms of crude carotene was agitated with 150 ml. of 12 per cent alcoholic potassium hydroxide for 5 minutes in the jar of a Waring Blendor at room temperature. Petroleum naphtha (Skellysolve F) was added, the solution diluted with water, and the crude carotene extracted with petroleum naphtha, purified by washing with methanol as in the A. O. A. C. method for crude carotene (*1*), dried with anhydrous sodium sulfate, and made up to volume. The crude carotene was determined with the photoelectric colorimeter, the solution was concentrated in a vacuum to about 10 or 15 ml., and the pigments were separated in a column of calcium hydroxide. The chromatographic technique employed was the same as has been previously described (*3*, *10*), except that commercial hydrated lime passed through a 65-mesh sieve was used instead of magnesium oxide. The bands of color were separated mechanically, the color was eluted from each separation with petroleum naphtha containing ethanol, the eluates were made up to volume, and the color was read in a KWSZ photoelectric colorimeter and calculated to carotene (*3*, *10*).

When this method was tested with solutions of purified crystalline carotene, or carotene in oil, no detectable amounts of any carotene isomers were found.

The analyses of a number of materials by this method are given in Table II. The crude carotene in some of the samples was also determined by the A. O. A. C. method (*1*) and found to be practically the same as by the modified method. The losses in the chromatographic analyses ranged from 0.4 to 20.3 per cent and were prorated so that the ingredients totaled 100 per cent (*3*, *10*). As shown in Table II, the composition of the carotene solutions varied widely according to the material used. No neo-beta-carotene was found in hegari silage, fresh carrots, and fresh turnip greens. In samples from other kinds of materials the quantities ranged from 2.3 to 18.1 per cent. Carrots contained 26 to 29 per cent of alpha-carotene, and very small amounts were found in some of the other samples. Impurity A, which includes several bands in the upper part of the column above carotenoid X, ranged from 2.8 per cent of the pigments with carrots to 39.5 per cent with sumac sorghum silage. Alfalfa meal contained from 5.8 to 30.8 per cent. Apricots contained 30.8 per cent of impurity A, a portion of which was lycopene. Carotenoid X, which formed a band just above the beta-carotene, ranged from 0 to 26.7 per cent. The beta-carotene was as low as 26.4 per cent in one sample of alfalfa and as high as 95.4 per cent in fresh turnip greens. The neo-beta-carotene band was below the beta-carotene and above the alpha-carotene band, when alpha-carotene was present.

It is desirable to know the quantity of the various constituents of carotene secured by the more widely used of the methods described for the determination of carotene. These methods were compared on carotenoid solutions all prepared by treatment with alcoholic potash and extraction with petroleum naphtha as for the analyses in Table II. The petroleum naphtha extracts were treated in the different ways, the pigment mixtures obtained by each treatment were separated by adsorption on calcium hydroxide, and the quantities of each were determined.

In method A, the carotenoid solution was purified with 90 per cent methanol as in the A. O. A. C. method (*1*). In method B, it was purified with dilute diacetone alcohol (6 parts of water and 100 parts of diacetone alcohol) as in the method of Hegsted, Porter, and Peterson (*8*). In method C, the extract after purification with methanol was shaken with activated magnesium carbonate (*4*). In method D, the extract was concentrated and placed on a short column of magnesium carbonate (*11*). The crude carotene was washed through the column and the impurities were adsorbed. Method E is the same as D, except that dicalcium phosphate was used (*12*). Method F is similar to D and E, except that the adsorbent consisted of 1 part of magnesium oxide and 3 parts of Supercel as specified by Wall and Kelly (*14*). After the pigments had been adsorbed, the crude carotene was eluted by

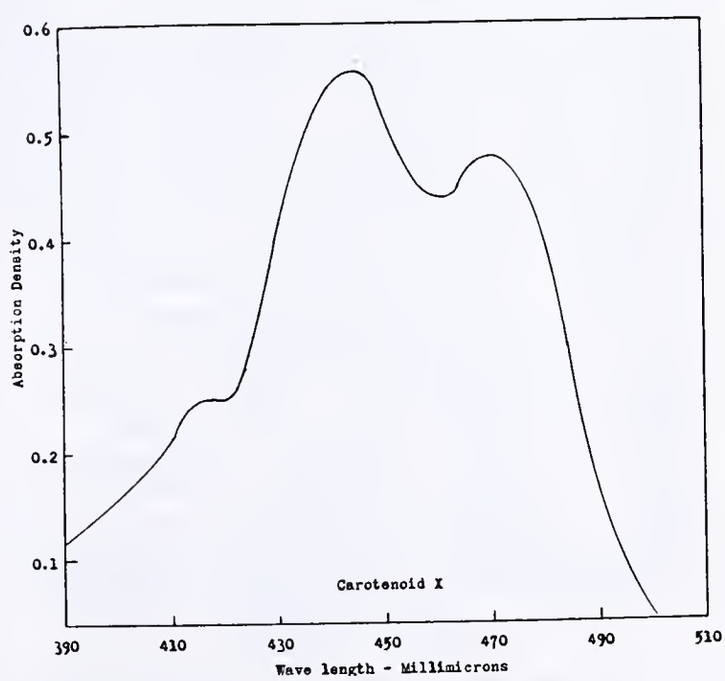

Figure 1. Absorption Curve of Carotenoid X in Petroleum Naphtha

Table III. Constituents of Carotene Solutions Obtained by Different Methods

Method of Purification	Crude Carotene	Loss in $Ca(OH)_2$ Columns	Constituents: Impurity A	Carotenoid X	Beta-carotene	Neo-beta-carotene	Alpha-carotene
	P. p. m.	%	%	%	%	%	%
Alfalfa, dehydrated							
A. Methanol	31.7	7.9	30.8	26.1	37.5	5.6	...
B. Diacetone	31.0	10.3	20.1	...	70.5	9.4	...
C. Methanol and shaking with X reagent	24.0	5.8	16.3	30.2	46.0	7.5	...
D. $MgCO_3$ column	23.0	0.9	18.4	30.7	41.3	9.6	...
E. $CaHPO_4$ column	22.0	1.8	12.5	34.3	44.4	8.8	...
F. MgO + Supercel column	22.0	15.8	9.2	19.8	60.3	10.7	...
G. 85% H_3PO_4	27.6	7.3	33.8	15.5	29.1	21.6	...
Alfalfa, dehydrated							
A. Methanol	105.0	10.5	14.9	20.2	46.8	18.1	...
B. Diacetone	96.0	9.9	12.1	19.7	55.5	12.7	...
C. Methanol and shaking with X reagent	88.0	3.4	4.7	15.3	60.0	20.0	...
D. $MgCO_3$ column	77.0	−0.6	1.3	13.6	60.6	24.5	...
E. $CaHPO_4$ column	77.0	8.4	2.1	14.9	52.5	30.5	...
F. MgO + Supercel column	71.0	8.8	8.3	23.8	47.7	20.2	...
G. 85% H_3PO_4	83.5	2.4	5.5	21.4	49.2	23.9	...
Canned spinach							
A. Methanol	38.8	2.6	25.1	21.6	35.4	17.3	0.6
B. Diacetone	40.4	9.4	15.8	31.7	37.2	13.7	1.6
C. Methanol and shaking with X reagent	37.5	1.1	5.1	21.5	53.2	18.0	2.2
D. $MgCO_3$ column	28.8	3.4	1.1	29.8	45.8	22.8	0.5
E. $CaHPO_4$ column	34.4	4.1	5.1	21.8	44.9	26.1	2.1
F. MgO + Supercel column	36.2	5.8	1.8	22.9	51.6	19.9	3.8
G. 85% H_3PO_4	29.5	9.2	3.0	23.9	47.7	25.4	...
Canned pumpkin							
A. Methanol	25.8	2.7	19.9	...	46.6	9.6	23.9
B. Diacetone	26.2	10.3	12.0	16.2	30.2	19.4	22.2
C. Methanol and shaking with X reagent	22.8	0.0	4.4	5.7	47.3	7.5	35.1
D. $MgCO_3$ column	23.2	1.7	3.5	7.5	43.8	11.9	33.3
E. $CaHPO_4$ column	20.6	−2.9	1.9	6.1	48.2	13.7	30.1
F. MgO + Supercel column	21.8	3.7	5.7	11.4	40.9	17.3	24.7
Dehydrated carrots							
A. Methanol	844.0	10.6	3.9	...	63.4	4.9	27.8
B. Diacetone	840.0	17.4	5.3	...	59.6	5.8	29.3
C. Methanol and shaking with X reagent	853.0	12.9	2.7	...	63.7	4.5	29.1
D. $MgCO_3$ column	783.0	5.1	2.1	...	62.3	4.2	31.4
E. $CaHPO_4$ column	763.0	5.5	2.6	...	60.9	6.3	30.2
F. MgO + Supercel column	875.0	6.1	1.9	...	61.9	5.5	30.7

washing the column with petroleum ether containing 3 to 5 per cent acetone. The eluate was washed with water, concentrated in a vacuum, and subjected to chromatographic analysis. In method G the carotenoid extract was shaken with 85 per cent orthophosphoric acid [Haagen-Smit, Jeffreys, and Kirchner (*7*)].

The results given in Table III show that none of the methods of purification gives a pure carotene solution of biologically active pigments. The solution purified by methanol contained the highest percentage of impurity A. The solution purified by diacetone came next. Phosphoric acid removes considerable amounts of impurity A from two solutions but none from a third. All the other methods which involve adsorbents remove appreciable portions of impurity A, but not all of it. The different methods of purification had little effect upon the quantities of carotenoid X. In some cases there was an apparent increase in this pigment, but since the results are based upon percentages, a decrease in impurity A would result in an increase in other constituents. The dehydrated carrots contained from 0.9 to 5.0 per cent of a pigment which appeared to be neo-alpha-carotene and is included in Table III as alpha-carotene.

The absorption curve of carotenoid X, prepared from canned spinach and dehydrated alfalfa (Figure 1), had strong maxima at 445 and 470 millimicrons and a slight maximum at 415 millimicrons. Biological analyses with rats by W. W. Meinke show that this pigment prepared from spinach does not possess vitamin A activity.

Discussion of Results

All methods for the determination of carotene gave solutions containing beta-carotene, neo-beta-carotene, impurity A, carotenoid X, and in a few cases alpha-carotene and neo-alpha-carotene. The relative proportions of the ingredients depended upon the material used and the method of purification. No method of purification had an appreciable effect upon the quantity of carotenoid X, which appears to be so closely related to beta-carotene that, like neo-beta-carotenes, it is separated only by chromatographic analysis with calcium hydroxide. The fact that carotenoid X lacks vitamin A potency makes it highly significant in evaluating the vitamin A activity of materials from carotene analyses. The neo-beta-carotene found is not so significant because according to biological tests with rats by W. W. Meinke in this laboratory, it has one half the potency of beta-carotene. Impurity A is also significant, in that it has very little, if any, vitamin A activity (*15*).

Adsorption on calcium hydroxide is a method available for the separation and determination of the various carotenes in the mixture obtained in the analysis of plant materials. This method, however, requires much time. There are some losses in the calcium hydroxide column which can be reduced by an experienced worker. For rapid control work, it will probably be desirable to determine crude carotene by a convenient method which at the same time gives values that closely approximate the actual amounts of carotenes which possess vitamin A activity. This crude carotene will contain colored impurities, and will also frequently contain carotenes which have lower biological values than beta-carotene. The carotene solutions secured from plants by any method of determination heretofore proposed, except chromatographic separation with calcium hydroxide, usually contain three or more yellow pigments, analysis by these methods must be recognized as giving only approximate results.

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Chemical and Physical Determinations of Vitamin A in Fish Liver Oils

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The results of a comparative study of spectrophotometric and colorimetric (antimony trichloride) methods for the estimation of vitamin A in fish liver oils are reported. An improved method for plotting the ultraviolet absorption curves of vitamin A products is presented, and applied in studies of crystalline vitamin A acetate, fish liver oils, and concentrates to evaluate factors which cause distortions in the curves. Emphasis is placed on the importance of conducting the determination on the unsaponifiable fraction of oils regardless of their potency. A method is described for extracting the unsaponifiable fraction without the loss of vitamin or the introduction of irrelevant absorbing materials. The U. S. P. reference cod liver oil No. 2 is shown to be unsuited as a spectrophotometric or colorimetric standard.

THE biological vitamin A activity of food or pharmaceutical products may result from the presence of a multiplicity of substances, including preformed vitamin A, either as the free alcohol or as esters, and various carotenoid pigments, of which the principal one is β-carotene. Obviously, therefore, the estimation by chemical or physical means of the vitamin A potency of a product may be complicated to a degree dependent on the nature and number of compounds present having vitamin A activity in vivo. A further factor outside the scope of nonbiological methods is the rate and degree of absorption from the intestinal tract of the various forms of vitamin A. This is dependent upon the cellular structure, digestibility, and fat and tocopherol content of the food, and is affected by the presence of nonabsorbable oils. Thus the true vitamin A potency of a food can be measured only biologically, whereas chemical or physical methods yield an estimate of the total content of vitamin A or its precursors.

The vitamin A potency of fish liver oils can be readily determined by spectrophotometric and colorimetric methods of assay, because in these oils the vitamin occurs almost exclusively as preformed vitamin A and is present in comparatively high concentrations.

Ultraviolet Absorption Curve of Free and Esterified Vitamin A

The ultraviolet absorption curve of pure vitamin A has appeared in several recent reports (*2*, *19*). In Figure 1 are plotted the critical parts of the curves (from 300 to 350 millimicrons) for isopropanol solutions of vitamin A acetate (obtained from Distillation Products, Rochester, N. Y.) and the alcohol prepared therefrom. For reasons stated below it is desirable to plot the results in terms of extinction ratios ($E_{\lambda/328}$) rather than extinction coefficients ($E^{1\%}_{1\,cm.}$ 328 mμ). Another pure vitamin A preparation, the distilled vitamin A esters (from Distillation Products) in corn oil, has been found to yield practically the same curves before and after saponification (*11*).

The Beckman quartz spectrophotometer (manufactured by National Technical Laboratories, South Pasadena, Calif.), calibrated against the lines of the mercury spectrum, was used for the spectrophotometric measurements. To determine the reproducibility of this instrument a series of glass disks, optically ground and having graded absorption properties, was prepared and distributed among various laboratories which were requested to measure their extinction coefficients at 328 millimicrons. The results of these tests indicated a high degree of reproducibility for the Beckman instrument, the coefficient of variation of the average deviations being of the order of 1 per cent. A recent study of several spectrophotometers used for assaying vitamin A oils (*15*) likewise showed excellent reproducibility in the Beckman measurements.

After saponification of the vitamin A ester a slight shift towards the lower wave lengths occurs in the entire absorption curve, so that the absorption maximum for the alcohol is at 325 rather than 327 millimicrons. This phenomenon has been observed not only with the pure compound, but also with naturally occurring fish liver oils. Another point of interest, illustrated in Table I, is the higher extinction coefficient at 325, or even 328 millimicrons, of an equivalent amount of the alcohol (prepared by saponification of the acetate) as compared with that of the acetate. Similar observations have been reported in studies on fish liver oils when the measurements were made in polar solvents, in which the unsaponifiable fraction gave a higher extinction coefficient than in nonpolar solvents (*1*, *8*). This was not so pronounced when the determinations were made on the whole oils, where the vitamin occurs esterified.

In Figure 1, and in all the absorption curves presented, emphasis is placed on the qualitative nature or shape of the absorption curve rather than on the absorption intensity or position on the vertical axis as determined by the concentration. The ordinate represents the extinction ratio—that is, the ratio of the extinction coefficient (or photometric density) at a given wave length to that at 328 millimicrons, the generally accepted absorption maximum for naturally occurring vitamin A esters. In order to demonstrate the advantages of this method of plotting the absorption curve, the same data for vitamin A acetate after oxidation and after dilution are plotted in Figure 2 in terms of extinction coefficients and extinction ratios. When plotted in terms of extinction coefficients, simple dilution reduces the height of the curve, as does oxidation, but no noticeable difference is seen in the shape of these curves. When the curves are plotted in terms of extinction ratios, the influence of oxidation, the presence of irrelevant absorbing materials, or other factors influencing the character of the curve become apparent. Ready comparison can then be made between the absorption curve of any sample, regardless of vitamin A content, and that of a sample of known purity—for example, the crystalline acetate. By supplementing

TABLE I. ULTRAVIOLET LIGHT ABSORPTION OF VITAMIN A ACETATE AND ALCOHOL[a]

Material	λ	Concentration in Isopropanol Solution	Photometric Density[b]
	mμ	%	
Vitamin A acetate	328	1.000	1570
Vitamin A alcohol	328	0.872[c]	1590
	325	0.872[c]	1620
	328	1.000	1825[d]
	325	1.000	1860[d]

[a] Same values were obtained on two samples of vitamin A acetate, before and after saponification, each assayed in duplicate. Measurements were made at 1-cm depth, after suitable dilution with isopropanol to bring test solution within proper range for spectrophotometric measurement.
[b] Equals (2 − $\log_{10} G$) × factor for dilution.
[c] Vitamin A alcohol obtained by saponification of 1.00 gram of vitamin A acetate (M. W. = 328).
[d] Expressed in terms of 1.000 gram of vitamin A alcohol derived from 1.146 grams of acetate.

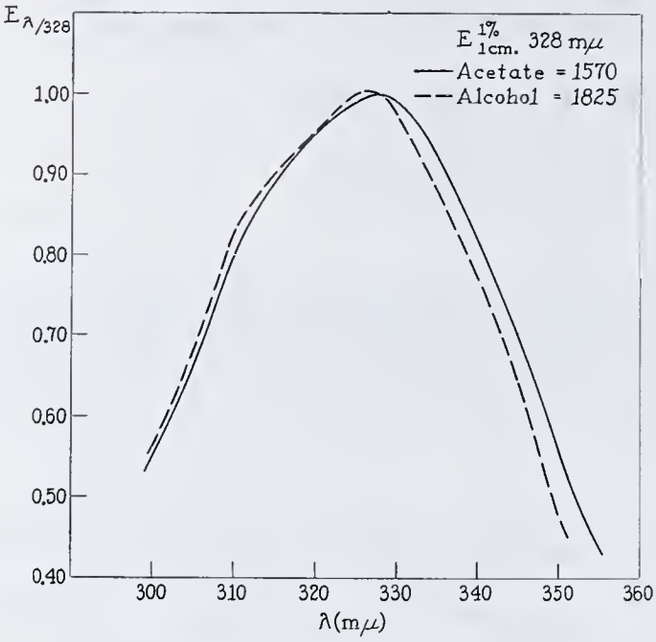

FIGURE 1. ULTRAVIOLET ABSORPTION CURVES VITAMIN A ACETATE AND VITAMIN A ALCOHOL IN ISOPROPANOL

the curve with the extinction coefficient at 328 millimicrons (the E value), the pertinent data for interpreting the spectrophotometric measurements are given.

Reaction of Vitamin A in Fish Liver Oils with Antimony Trichloride

This reaction measures vitamin A both as the ester and free alcohol. When the test is applied to fish liver oils directly, especially to low-potency oils, inhibitors of the color development are often encountered and must be corrected for by use of the increment procedure (*11*). Freshly opened samples of U. S. P. reference cod liver oil No. 2 appear to have large amounts of inhibitors. Upon saponification the inhibitors are removed and the vitamin develops its full color intensity with the reagent, as shown by a normal increment in photometric density for the 10 units of vitamin A added to an aliquot of the final test solution. In addition, nonspecific, saponifiable materials are present in fish liver oils which react with antimony trichloride to form a blue color absorbing light at 620 millimicrons. For these reasons it is preferable to use the unsaponifiable fraction rather than whole oil for test purposes.

Changes in the Absorption Curve of Vitamin A on Oxidation

Investigations of the oxidative destruction of vitamin A in fish liver oils, using various oxidation procedures and assay methods, have been reported (*6, 7, 13, 14, 17*). The results of a typical experiment to determine the effect of oxidation upon the ultraviolet absorption curve of crystalline vitamin A are presented in Figure 3.

Solutions of vitamin A acetate and vitamin A alcohol (obtained by saponification of the acetate) in ethyl laurate were vigorously aerated in parallel at 45° C., in the absence of light, in an all-glass apparatus. Samples were removed at intervals and their vitamin A content was determined spectrophotometrically and colorimetrically. In testing such pure solutions the precision of the two assay procedures is good; the spectrophotometric values are reproducible within ±1 per cent, the colorimetric within ±2 per cent. The air was passed through pure ethyl laurate, also at 45° C., before entering the test solutions, the diluent so aerated being used as a blank in the spectrophotometric measurements. Ethyl laurate is ideally suited for this type of experiment, since it absorbs light to a negligible extent in the range of 295 to 355 millimicrons, undergoes no optical changes upon aeration, and does not interfere with the color reaction with antimony trichloride, before or after aeration.

In the cases of both the alcohol and acetate the changes in the curve were limited almost entirely to the region below 328 millimicrons, the extinction ratios of the samples at the lower wave lengths increasing progressively during the period of oxidation. Robinson (*13*), in tests conducted with a fish liver concentrate rather than the pure vitamin, obtained similar results in so far as the spectrophotometric data are concerned. This is not readily apparent in his paper, since his curves were plotted in terms of extinction coefficients.

In these tests the inability of the antimony trichloride reagent to differentiate between oxidized and nonoxidized vitamin A is demonstrated. At first, there is fairly good agreement between the spectrophotometric and colorimetric estimates, as would be expected when working with pure solutions of vitamin A. [For comparison the colorimetric values are expressed as "vitamin A units per gram ÷ 2000", since the spectrophotometric estimates are derived from E value × 2000 (the commercial standard conversion factor).] During the initial stages of oxidation there is a tendency for the colorimetric values to be somewhat less than the E values. The slightly lower values obtained colorimetrically for the freshly prepared solutions may be due to small amounts of oxidized vitamin in the crystalline preparation. Large losses of vitamin A by oxidation must occur before significant distortions in the ultraviolet absorption curves become apparent. After prolonged aeration of the solutions, the values determined by the antimony trichloride reaction became equal to and then significantly greater than those obtained spectrophotometrically. Estimates of residual nonoxidized vitamin A based on the E values of the aerated solutions would, from the very nature of the absorption curves, be much too high. Colorimetric estimates approximating these values would thus also be in error. These observations do not agree with those of Robinson (*13*), who found that as the oxidation progressed the relative differences

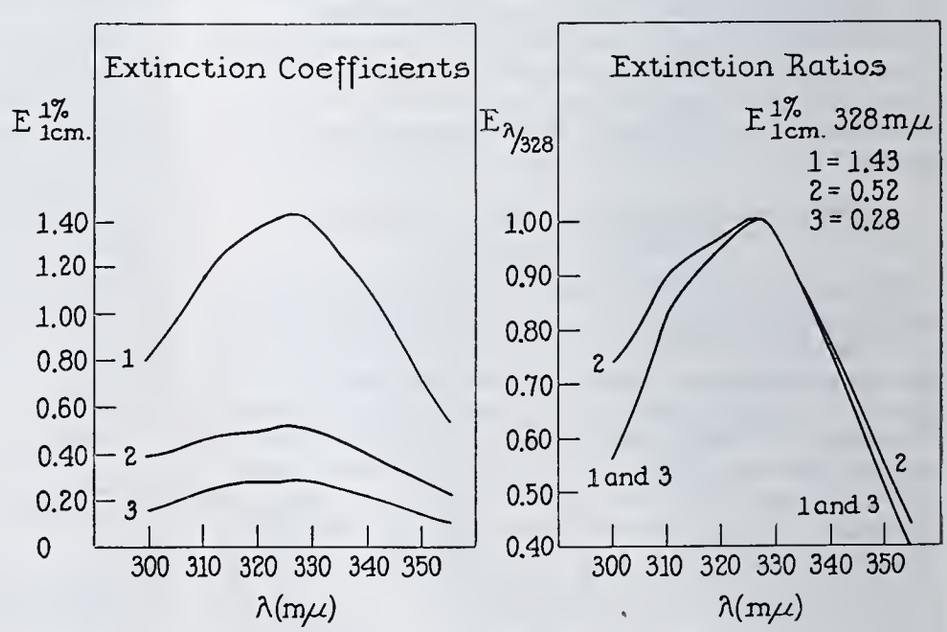

FIGURE 2. ADVANTAGE OF PLOTTING EXTINCTION RATIOS RATHER THAN EXTINCTION COEFFICIENTS IN PRESENTING ULTRAVIOLET ABSORPTION CURVES OF VITAMIN A SOLUTIONS

1. Vitamin A acetate in ethyl laurate
2. Oxidation of (1) by air
3. Dilution of (1) 1 to 5 with ethyl laurate

Table II. Ultraviolet Absorption Maximum of Samples of Fish Liver Oils[a]

(Fish liver oils taken at random from a series of test samples; values are not necessarily representative of each species.)

Sample	$E^{1\%}_{1\,cm.}$ 328 mμ	Maximum mμ	$E^{1\%}_{1\,cm.}$ Max.	Difference[b] $E^{1\%}_{1\,cm.}$ 328 mμ − $E^{1\%}_{1\,cm.}$ Max. %
Vitamin A acetate	1570	327	1570	0.0
Same, after saponification	1590	325	1620	−1.9
Distilled vitamin A esters	107.8	328	107.8	0.0
Same, unsaponifiable fraction	106.3	325	107.0	−0.7
Cod liver oil (No. 1)	0.87	320	0.94	−7.4
Same, unsaponifiable fraction	0.71	325	0.72	−1.4
Cod liver oil (No. 2)	1.58	328	1.58	0.0
Cod liver oil (No. 3)	1.14	320	1.27	−10.2
Same, unsaponifiable fraction	0.98	325	0.99	−1.0
Dogfish liver oil	6.04	328	6.04	0.0
Shark liver oil	8.02	326	8.11	−1.1
Soupfin shark liver oil	56.0	328	56.0	0.0
Black cod liver oil	55.2	328	55.2	0.0
Ling cod liver oil	94.1	328	94.1	0.0
Halibut liver oil	29.5	327	30.0	−1.6
Tuna liver oil	60.1	327	60.1	0.0
Concentrates (prepared by saponification)	56.7	326	56.9	−0.4
	96.1	327	97.2	−1.1
	498	325	508	−2.0

[a] In isopropanol solution.
[b] Value for $E^{1\%}_{1cm.}$ maximum is taken as basis for comparison.

between the spectrophotometric and colorimetric values increased, the colorimetric values always being lower.

As the oxidation proceeded the color reaction, with antimony trichloride became increasingly atypical. The rate of color development decreased; fully 30 seconds were required to reach maximal color intensity in the sample aerated for 140 hours, as compared with 4 seconds in the case of the non-oxidized solution. In measuring the color developed, the 4-second galvanometer reading was used rather than that corresponding to maximal color development. When the latter was used low values were obtained for added increments of vitamin A, resulting in extremely high vitamin A estimates for samples containing oxidized vitamin A.

The above colorimetric and spectrophotometric tests suggest that there may be more than one form of oxidized vitamin A, those first produced reacting to a lesser extent than vitamin A with the antimony trichloride reagent, while other compounds formed in the later stages of oxidation react to an even greater extent than vitamin A, based upon comparable E values at 328 millimicrons. Obviously the reaction of the latter forms of oxidized vitamin A with the antimony trichloride reagent is much less than that observed for an equivalent amount of the vitamin.

Reliability of Spectrophotometric Method Based upon a Single Reading at 328 Millimicrons

In determining the vitamin A potency of fish liver oils, there is, as yet, no complete agreement as to the wave length at which the extinction coefficient of the oil should be measured. The absorption maximum of vitamin A alcohol in polar solvents (such as isopropanol) is now known to be closer to 325 than to 328 millimicrons. However, fish liver oils may exhibit other absorption maxima in the critical region of the spectrum.

In Table II are presented extinction coefficients at both the maximum and 328 millimicrons of several fish liver oils chosen at random from a large series of samples. Excluding atypical oils which show absorption maxima far from that of vitamin A (usually low-potency oils), the samples are seen to have maxima ranging from 325 to 328 millimicrons in isopropanol. The unsaponifiable extracts of these same samples invariably exhibited maximal absorption at 325 millimicrons. It is apparent that, in fish liver oils with absorption maxima in the region of 325 to 328 millimicrons, the accuracy of a determination is not seriously affected by using the extinction coefficient at 328 millimicrons in preference to that at the maximum, or vice versa. The difference is of the order of 1 to 2 per cent. When the absorption maximum of an oil departs significantly from the 325 to 328 millimicron range, the presence of appreciable quantities of irrelevant absorbing material is indicated and vitamin A estimates based upon such a maximum are in error.

It is the practice in these and most other laboratories to evaluate the vitamin A content of fish liver oils on the basis of E values—i. e., the extinction coefficient at 328 millimicrons. It has been reasonably suggested that, in the case of unsaponifiable concentrates of fish liver oils, the extinction coefficient at 325 millimicrons be used. However, many such concentrates are frequently diluted with whole fish liver oils, a practice which introduces complications in assaying such materials. In any case, the use of the 328-millimicron

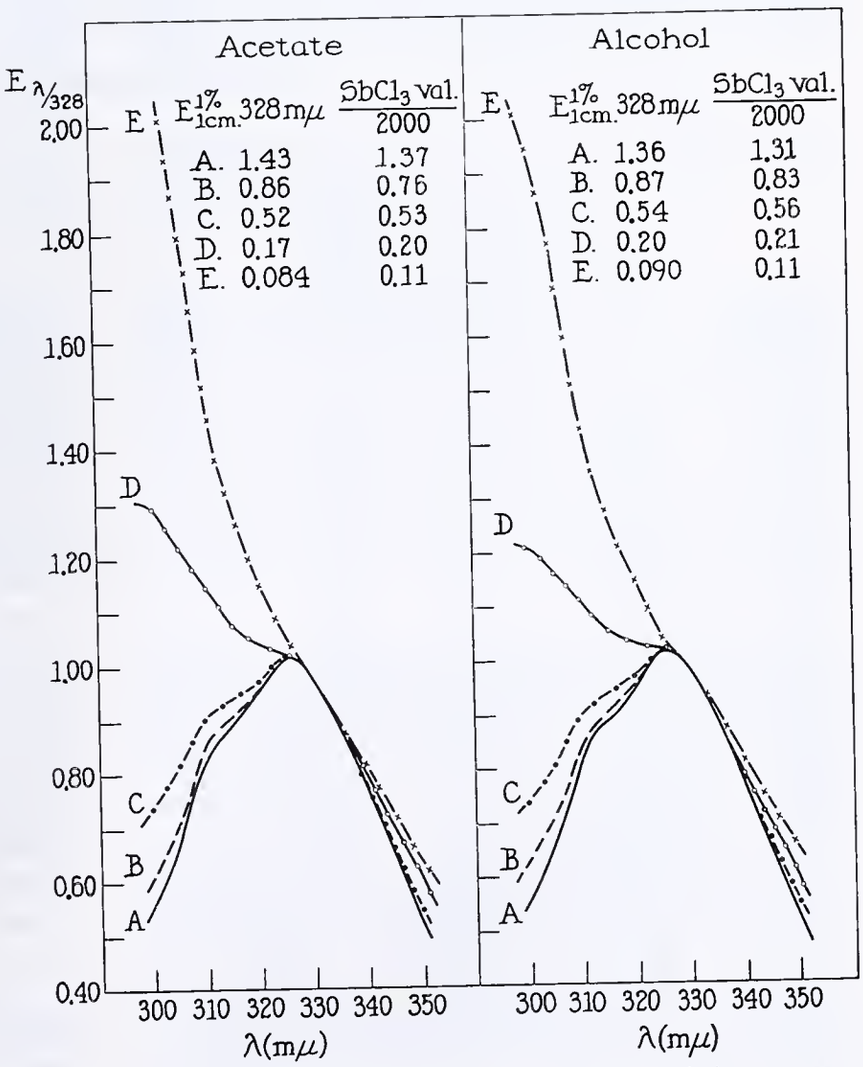

Figure 3. Changes in Absorption Curves of Vitamin A Acetate and Alcohol on Oxidation

Aerated at 45° C. *A*, 0 hour; *B*, 24 hours; *C*, 48 hours; *D*, 90 hours; *E*, 140 hours

Table III. Extraction of Unsaponifiable Fraction of Fish Liver Oils

(Step-by-step comparison of three methods)

Step	United States Pharmacopoeia XII (*12*)	Kerr-Sorher (Modified, *5*)	British Pharmacopoeia (Modified, *3*)
Saponification	Reflux 5 grams of oil in 40 ml. of 1.1 *N* alcoholic (95%) KOH for 120 minutes	Reflux 5 grams of oil in 35 ml. of 1.8 *N* alcoholic (86%) KOH for 20 minutes	Reflux 0.2 to 1 gram of oil in 15 ml. of 0.5 *N* alcoholic (95%) KOH for 15 minutes
Extraction	Evaporate the alcohol Take up the residue in 50 ml. of water Extract twice with 50 ml. of ether	Cool to 30° C. Extract once with 150 ml. of ether	Add 30 ml. of water Extract 4 to 5 times with 30 ml. of ether (Test last extract with $SbCl_3$)
Washing	Once with 20 ml. of 0.1 *N* NaOH Once with 20 ml. of 0.2 *N* NaOH Then with 20-ml. portions of water until the last washing gives no color with phenolphthalein	3 times with 100 ml. of 0.2 *N* KOH Then with 30-ml. portions of water until last washing gives no color with phenolphthalein	Once with 30 ml. of water Once with 15 ml. of 0.5 *N* KOH Then with 30-ml. portions of water until last washing gives no color with phenolphthalein
Evaporation	To dryness on a steam bath	To dryness on a steam bath	To *ca.* 20 ml. on a steam bath Remove from steam bath, and blow off the remainder of the solvent (do not bring to complete dryness) with a stream of nitrogen

Table IV. Reliability of Procedure for Preparation of Unsaponifiable Extract of Fish Liver Oils

Sample No.[a]	Procedure for Estimating Vitamin A	Vitamin A Content, U. S. P. Units per Gram: Whole sample, *a*	Unsaponifiable fraction, *b*	Resaponification: *b* alone	*b* + cottonseed oil
16	Spectrophotometric[b]	32,400	30,400	31,500	32,760
	$SbCl_3$ reaction[c]	32,300	29,600	32,200	30,500
18	Spectrophotometric	49,400	44,800	44,100	44,300
	$SbCl_3$ reaction	50,800	39,100	39,300	39,900
19	Spectrophotometric	52,000	50,000	49,600	49,600
	$SbCl_3$ reaction	52,900	42,800	43,100	47,200
22	Spectrophotometric	117,000	113,600	112,400	112,400
	$SbCl_3$ reaction	119,000	110,000	108,000	105,000
23	Spectrophotometric	120,800	111,400	114,500	114,500
	$SbCl_3$ reaction	122,000	117,000	117,000	114,000

[a] Numbers correspond to those in Table V.
[b] Commercial standard conversion factor of 2000 was used for estimating vitamin A content from $E^{1\%}_{1cm.}$ 328 mμ.
[c] Standard was a sample of distilled vitamin A esters; $E^{1\%}_{1cm.}$ 328 mμ (in isopropanol) = 108.5.

maximum yields values for most oils which are not significantly different from those calculated from their actual maxima.

Saponification Procedure

Because natural oils often contain materials which absorb nonspecifically in the ultraviolet region where vitamin A measurements are made, it is often necessary to separate the vitamin A by taking advantage of its nonsaponifiability, since the substances responsible for irrelevant absorption are mainly in the saponifiable fraction. The procedure for the extraction of the unsaponifiable fraction of fish liver oils as a step preliminary to the estimation of vitamin A content varies in different laboratories (*8*, *9*, *16*). In Table III is presented a comparison of the more common analytical procedures used for the determination of the unsaponifiable matter in oils.

The authors have employed a modification of the method described in the British Pharmacopoeia (*3*), using a sufficient number of extractions to ensure the complete extraction of all vitamin A, as indicated by a spot-plate test of the last extract with antimony trichloride. To prevent oxidation of the vitamin during the evaporation of the last few milliliters of ether, the beaker is removed from the steam bath and the last traces of solvent are removed by a stream of nitrogen. All operations are performed in amber-colored glassware (*4*).

A series of tests was conducted to find out whether any vitamin A is lost, either mechanically or through oxidation, in the course of the preparation of the unsaponifiable fraction. The results are shown in Table IV. Five samples of fish liver oil were saponified and their unsaponifiable fractions extracted as above. Three aliquots of each of the final ether extracts were evaporated down, with the precautions stated above. One of the residues was diluted in cottonseed oil to a concentration approximating that of the original whole oil, and was then treated as a fresh sample. Another was submitted to a repetition of the saponification and extraction procedures without the addition of cottonseed oil. The third residue was taken up in isopropanol directly and its extinction coefficient at 328 millimicrons measured.

If loss of vitamin A had occurred due to incomplete extraction or to oxidation, the vitamin A content of the second unsaponifiable fraction would be expected to be lower than that of the original unsaponifiable fraction. That such was not the case, however, may be seen in the good agreement for each sample among all three spectrophotometric estimates based on the unsaponifiable fractions. Similarly close agreement may also be observed where the vitamin content was determined by the antimony trichloride method. (Both the spectrophotometric and colorimetric tests were performed using aliquots of the same final ether extract.) A comparison of the ultraviolet absorption curves of the unsaponifiable fractions within a set showed no differences, a further indication that no substantial oxidative changes had occurred.

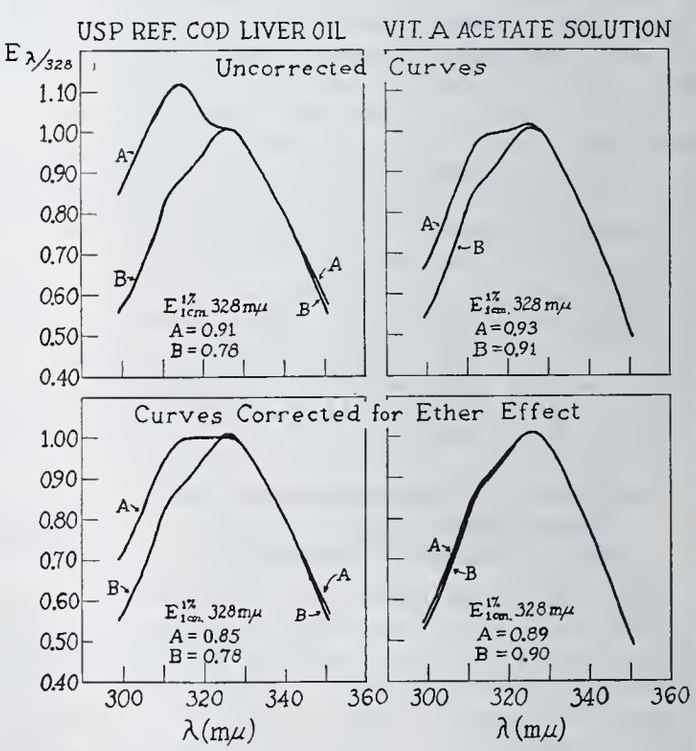

Figure 4. Influence of Impurities in Ether Used in Preparation of Unsaponifiable Extracts on Ultraviolet Absorption Curves and Extinction Coefficients of Vitamin A Alcohol Solutions

A. Preparation of unsaponifiable extract with U. S. P. ether
B. Preparation of unsaponifiable extract with redistilled U. S. P. ether

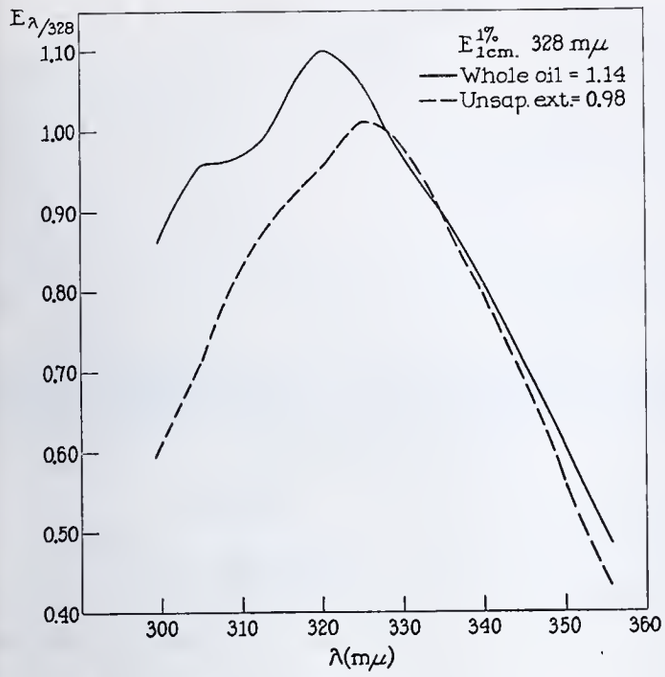

FIGURE 5. REMOVAL OF IRRELEVANT LIGHT-ABSORBING MATERIALS IN A COD LIVER OIL BY SAPONIFICATION

Increasing specificity of spectrophotometric method for determination of vitamin A

The necessity for the use of specially prepared alcohol for the saponification procedure and specially prepared ether for the subsequent extraction was investigated. The general practice has been to use aldehyde-free alcohol and peroxide-free ether. When alcohol (S. D. No. 30) and ether (U. S. P. XII) were used directly from their commercial containers in the preparation of the unsaponifiable extracts of low potency oils, erroneously high extinction coefficients at 328 (or 325) millimicrons were obtained. Furthermore, the ultraviolet absorption curves were atypical of vitamin A alcohol.

A series of assays was conducted on the U. S. P. reference cod liver oil No. 2 on pure vitamin A acetate in 95 per cent ethyl alcohol (aldehyde-free), and on a sample of distilled vitamin A esters. A suitable sized sample of each was saponified using (*a*) redistilled alcohol (over aluminum and potassium hydroxide) and nonredistilled ether, (*b*) redistilled alcohol and redistilled ether, (*c*) nonredistilled alcohol and nonredistilled ether, and (*d*) nonredistilled alcohol and redistilled ether. The ether was redistilled without the addition of any reagents, discarding the first and last 10 per cent of the distillate.

The completeness of the removal of the aldehydes from the alcohol was verified by the absence of any color development on the addition of fuchsin-sulfite reagent (*18*) which produced an intense red color with the untreated solvent.

The use of nonredistilled alcohol affected neither the extinction coefficient nor the shape of the absorption curve. On the other hand, the use of nonredistilled ether resulted in both a distorted curve and a high extinction coefficient at 328 millimicrons. In Figure 4 (upper two sets of curves) are presented the absorption curves of the unsaponifiable fractions of the U. S. P. reference cod liver oil and that prepared from an ethanol solution of vitamin A acetate. The effect of using nonredistilled ether is apparent.

To correct for the ether effect, the same volume of ether used in the above tests was evaporated under the same conditions, and the residue was dissolved in the same volume of isopropanol. The absorption curve was then obtained. A similar correction was made for the redistilled ether, but this was extremely small. On applying these corrections the lower set of curves in Figure 4 was obtained. In the case of the vitamin A acetate both curves became superposable, indicating that the vitamin A itself was not affected by the impurities in the ether, which are carried through the saponification procedure to the final solution. In the case of the U. S. P. oil, only partial improvement was noted. Apparently, substances in this oil other than vitamin A had been affected.

The ultraviolet absorption curves (and likewise the extinction coefficients at 328 millimicrons) of the unsaponifiable extracts of the distilled vitamin A esters were the same regardless of which set of solvents was used. This is undoubtedly due to simple dilution of the nonspecific absorbing substances in the ether when adjusting the vitamin A concentration of the test solution to the proper range for the photometric measurements. In this case also the vitamin A was not affected.

Necessity for Determining the Vitamin A Content in the Unsaponifiable Fraction

Large errors may be made in the determination of the vitamin A content of fish liver oils when the estimates are based on a whole oil rather than on its unsaponifiable fraction. It is generally agreed that in the low-potency ranges these differences are significant. In Figure 5 is illustrated the change in the ultraviolet absorption curve in isopropanol of a sample of cod liver oil after saponification. The whole oil has two maxima, neither of which is characteristic of vitamin A. Furthermore, the extinction coefficient at 328 millimicrons is 16 per cent higher for the whole oil than for its unsaponifiable fraction.

On the other hand, it is not so commonly recognized that differences of equally important magnitude may be observed even in high-potency oils. Morton (*10*) states, "In the case of oils containing more than 10,000 I. U. per gram it is rarely necessary to resort to extraction of the nonsaponifiable fraction, because at 325 millimicrons, irrelevant absorption is a negligible fraction of the

TABLE V. COMPARISON OF $E^{1\%}_{1\,cm.}$ 328 mμ OF WHOLE OILS AND THEIR UNSAPONIFIABLE FRACTIONS

(In oils widely varying in vitamin A potency)

Sample No.	$E^{1\%}_{1\,cm.}$ 328 mμ Whole oil	$E^{1\%}_{1\,cm.}$ 328 mμ Unsaponifiable fraction	Difference[a] %	Difference Expressed as Vitamin A Unitage *U. S. P. units per gram*
1	0.23	0.07	−68.2	−320
2	0.87	0.53	−39.8	−680
3	0.91	0.57	−37.9	−680
4	0.93	0.91	−2.2	−40
5	1.12	1.05	−6.5	−140
6	1.14	0.98	−14.0	−320
7	1.49	0.87	−41.6	−1,240
8	1.51	1.20	−20.8	−620
9	1.60	1.63	+2.0	+60
10	1.65	1.52	−7.6	−260
11	1.75	1.43	−18.6	−640
12	4.89	4.65	−4.9	−480
13	5.73	5.59	−2.4	−280
14	8.25	8.26	+0.1	+20
15	9.44	9.53	+1.0	+180
16	16.2	15.2	−6.2	−2,000
17	23.9	21.5	−10.1	−4,800
18	24.7	22.4	−9.3	−4,600
19	26.0	25.0	−3.8	−2,000
20	32.5	32.6	+0.3	+200
21	41.9	39.3	−6.3	−5,200
22	58.5	56.8	−2.9	−3,400
23	60.4	55.7	−7.8	−9,400
24	61.7	60.7	−1.7	−2,000
25	64.3	59.9	−6.8	−8,800
26	72.8	69.9	−3.9	−5,800
27	96.6	92.6	−4.1	−8,000
28	99.8	99.0	−0.8	−1,600
29[b]	108.5	107	−1.4	−3,000
30	179	171	−4.5	−16,000
31	198	187	−5.5	−22,000
32	606	580	−4.3	−52,000
33	658	617	−6.2	−82,000

[a] Values obtained for whole oils are used as basis for comparison. Standard commercial conversion factor (2000) was used for estimating vitamin A content from $E^{1\%}_{1\,cm.}$ 328 mμ.

[b] Distilled vitamin A esters.

observed value. If, however, the color test appears anomalous, or does not agree with the ultraviolet assay, it is always safer to work on the nonsaponifiable fraction." However, even when good agreement by both methods is obtained, vitamin A estimates of high-potency oils may be appreciably less when the determination is conducted on the unsaponifiable fraction. In Table V are shown the differences in spectrophotometric estimates of vitamin A content between the whole and unsaponifiable fractions of a series of oils ranging from low to high potencies. A similar tabulation, using the antimony trichloride procedure (*11*), is shown in Table VI.

In addition to greater accuracy, an increase in precision is gained by conducting the antimony trichloride test according to the method described (*11*). The average deviation in the assay of fish liver oils (unsaponifiable fractions) is ordinarily of the order of ±2 per cent, which compares favorably with that of the spectrophotometric method. This may be illustrated by the data in Table IV where, even if the more involved redeterminations of the unsaponifiable fractions be regarded as triplicate tests, the average deviation by the spectrophotometric method is found to be ±1.04 per cent, and by the colorimetric method it is ±2.19 per cent. When duplicate determinations differ by more than ±2 per cent of their mean, a redetermination is indicated.

It has already been shown that the lower values are not due to mechanical or oxidative losses in the preparation of the unsaponifiable extracts. The possibility exists, however, that vitamin A degradation products, which absorb light at 328 millimicrons, are partially or completely removed by virtue of an appreciable solubility in the aqueous solutions used in the preparation of the unsaponifiable extract. Accordingly, an investigation along these lines was carried out.

An ethyl laurate solution of vitamin A acetate was aerated according to the procedure previously described. Samples were removed at intervals and spectrophotometric and colorimetric assays were performed on the whole sample and on the unsaponifiable fraction derived therefrom. The results of this experiment are presented in Figure 6. The rate of oxidative destruction of vitamin A in this experiment was more rapid than that in the previous aeration experiment, doubtless because of the more rapid rate of aeration of the present solution of the vitamin A acetate. This, however, does not introduce a variable into the study, since the aeration rates were maintained constant within each experiment, and all comparisons were made relative to the freshly prepared solutions. In each case, the rate of aeration during the progressive stages of oxidation of any particular set or sets within a single experiment was maintained constant.

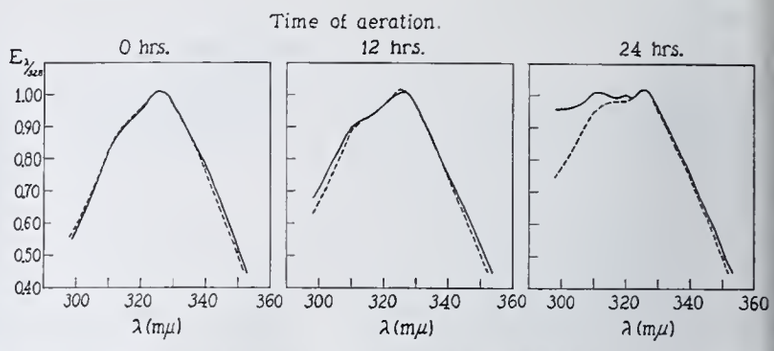

Figure 6. Improvement in Ultraviolet Absorption Curve on Saponification (Broken Line) of an Ethyl Laurate Solution of Crystalline Vitamin A Acetate (Solid Line) Subjected to Progressive Oxidation

Period of aeration, hours	0	12	24
		$E_{1\,cm.}^{1\%}$ 328 mμ	
Whole sample	2.40	1.28	0.72
Unsaponifiable extract	2.39	1.26	0.68
		$SbCl_3$ Value ÷ 2000	
Whole sample	2.21	1.17	0.63
Unsaponifiable extract	2.26	1.20	0.64

The data presented in Figure 6 show that the *E* value of the unsaponifiable fraction was significantly less than that of the whole sample when the latter was subjected to prolonged aeration. The initial test solution contained no appreciable amounts of irrelevant absorbing material, since it was a pure ethyl laurate solution of crystalline vitamin A acetate. Hence it appears that the oxidation products of vitamin A are partially removed in the preparation of the unsaponifiable fraction.

This is further supported by the character of the ultraviolet absorption curves obtained for the sample during progressive oxidation. Initially the curves for both the whole sample and unsaponifiable fraction corresponded with those for vitamin A acetate and vitamin A alcohol. During oxidation of the vitamin A ester the curve became progressively distorted owing to the presence of increasing amounts of vitamin A oxidation products. On the other hand, the absorption curves of the unsaponifiable fractions of the oxidized samples approached more closely that of the pure vitamin A alcohol. Obviously some oxidation products were removed in preparing the unsaponifiable extract. Since an atypical curve was still obtained with the unsaponifiable fraction it is concluded that not all of the oxidation products are removed by this procedure. Thus, estimates of the vitamin A potency of an oxidized oil (high- or low-potency) when based upon the whole sample may be erroneously high. The unsaponifiable fraction should be used for the test whenever possible.

The excellent agreement between the whole and unsaponifiable fractions of the freshly prepared vitamin A acetate solution with respect to both *E* values and colorimetric values (see legend to Figure 6) is further evidence of the reliability of the procedure used in the present study for the preparation of the unsaponifiable extract.

The excellent agreement between the colorimetric tests on the whole sample and on the unsaponifiable fraction appears at first to be somewhat at variance with the results obtained in the assay of fish liver oils (see Table VI). Apparently, the substances present in fish liver oils which react with the antimony trichloride reagent and are removed in the preparation of the unsaponifiable extracts, are not simply oxidized vitamin.

Table VI. Comparison of Vitamin A Values for Whole Oils and Their Unsaponifiable Fractions

(By the antimony trichloride reaction)

Sample No.[a]	$SbCl_3$ Value ÷ 2000[b] Whole oil	$SbCl_3$ Value ÷ 2000[b] Unsaponifiable fraction	Difference[c]	Difference in Vitamin A Content[c]
	Per gram	Per gram	%	U. S. P. units per gram
5	0.91	0.88	−3.3	−60
7	1.11	0.57	−48.6	−1,080
11	1.14	1.07	−6.1	−140
16	16.2	14.8	−8.6	−2,800
17	24.4	19.6	−19.6	−9,600
18	25.4	19.6	−22.8	−11,600
19	26.5	21.4	−19.2	−10,200
20	33.9	29.8	−12.1	−8,200
22	59.5	55.0	−7.6	−9,000
23	61.0	58.5	−4.1	−5,000
25	63.0	60.0	−4.8	−6,000
26	68.5	60.5	−11.7	−16,000
27	91.5	85.0	−7.1	−13,000
29[d]	108.5	107	−1.4	−3,000
30	179	154	−14.0	−50,000
31	193	178	−7.8	−30,000
32	605	490	−19.0	−230,000
33	660	580	−12.1	−160,000

[a] Numbers correspond to those in Table V.
[b] Standard used in these tests was a sample of distilled vitamin A esters; $E_{1\,cm.}^{1\%}$ 328 mμ (in isopropanol) = 108.5. To facilitate comparisons with data presented in Table V, vitamin A values obtained colorimetrically are divided by standard conversion factor, 2000.
[c] Values obtained for whole oil are taken as basis for comparison.
[d] Distilled vitamin A esters.

U. S. P. Reference Cod Liver Oil No. 2

The U. S. P. reference cod liver oil has many disadvantages as a reference standard in spectrophotometric and colorimetric work, some of which have been pointed out (*8*). One of the most important is the large difference in extinction coefficient at 328 millimicrons between the whole oil and its unsaponifiable fraction. Another is the lack of agreement between the spectrophotometric and colorimetric vitamin A values.

A series of five U. S. P. reference oils, obtained during the period from January to April, 1943, was found to have *E* values ranging from 0.88 to 0.92 (average = 0.90) for the whole oil and from 0.77 to 0.82 (average = 0.79) for the unsaponifiable fraction. (These tests were conducted on samples taken only from freshly opened bottles.) These differences were of the order of 12 to 14 per cent. According to the colorimetric method (*11*) the potency of the reference oils varied from 1400 to 1480 U. S. P. units per gram (average = 1450) for the whole oil and from 1310 to 1410 units per gram (average = 1360) for the unsaponifiable fraction, a difference of 7 per cent. These differences between the values for the whole oil and the unsaponifiable fraction assume added significance when it is considered that the method described in the U. S. Pharmacopoeia XII (*12*, pp. 329–30) for determining the spectrophotometric absorption value for cod liver oil does not specify which should be taken for test purposes. Failure to include such a specification is tantamount to assuming that this relation between the absorption values for whole oil and unsaponifiable fraction is uniform for all oils, which of course is not supported by the facts.

When parallel tests on the reference oil are used as the basis for arriving at factors for converting *E* values to biological unitage in unknown oils, serious complications result. Errors in estimating potency in relation to the U. S. P. reference oil may fall within the limits of error of the bioassay and are difficult to prove.

Another disadvantage in the use of the U. S. P. reference oil is its chemical instability. Bottles of the oil were obtained directly from the U. S. Pharmacopoeial Convention, Philadelphia, Penna., and the spectrophotometric absorption curves of the whole oil and unsaponifiable fraction were determined before and after storage for one month at 5° C. under air or nitrogen. A typical set of curves is shown in Figure 7.

Changes not only in the extinction coefficient at 328 millimicrons but also in the actual shape of the absorption curves are noted. The distortion of the curve of the stored sample is more striking in the case of the whole oil. The upward shift of the left leg of the vitamin A curve and the decrease in extinction coefficient at the absorption maximum is typical of an oxidative change. In the test conducted on the unsaponifiable fraction it is of interest to note the slight rise in *E* value of the sample stored under air. This is accompanied by an upward shift of the left leg of the curve. Such a shift in the maximum and the rise in *E* value is not infrequently observed in the incipient stages of oxidation of an oil. Comparison of the absorption curve of the unsaponifiable fraction of the fresh sample with that of pure vitamin A indicates that even this fraction contains irrelevant absorbing material. In each series that portion of the ultraviolet absorption curves above 330 millimicrons is superposable.

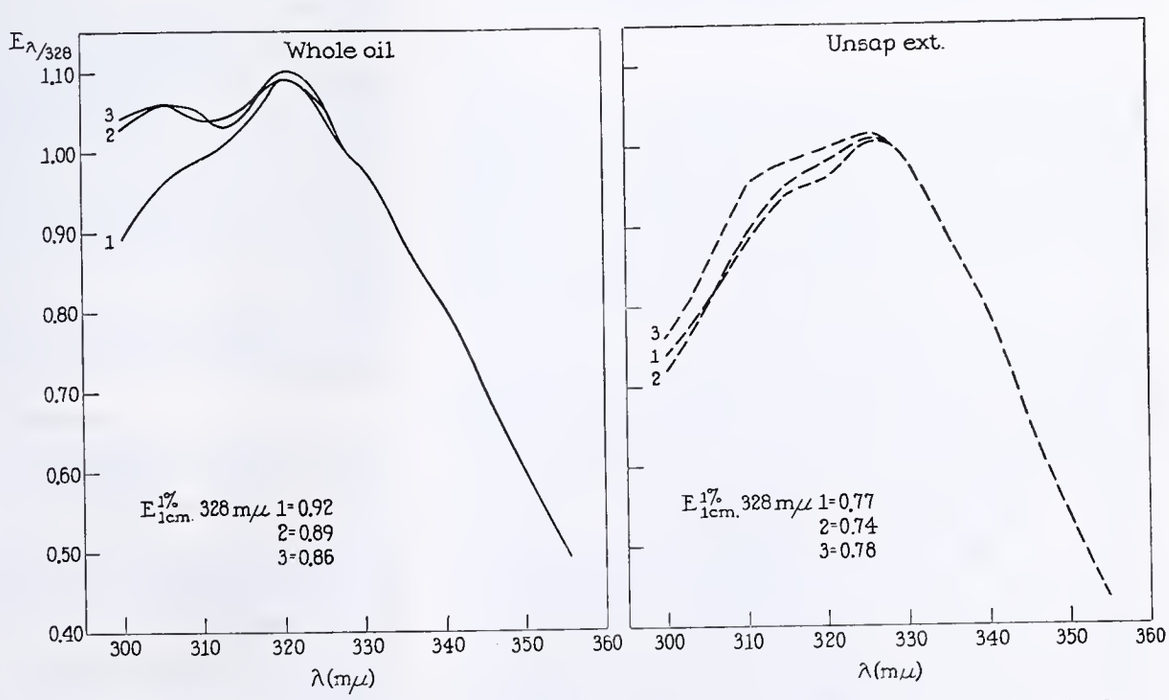

FIGURE 7. ULTRAVIOLET ABSORPTION CURVES OF U. S. P. REFERENCE COD LIVER OIL NO. 2

Before (curve 1) and after storage at 5° C. under nitrogen curve 2) and air (curve 3)

Summary

The spectrophotometric and colorimetric (antimony trichloride) methods for the determination of vitamin A in fish liver oils have been critically studied.

The ultraviolet absorption curves for crystalline vitamin A acetate and the alcohol, derived therefrom, are presented. By plotting extinction ratios rather than extinction coefficients it is possible to obtain curves for oils, regardless of vitamin A content, which lend themselves to direct comparison with that of a pure vitamin A preparation. During oxidation of vitamin A, either as the free alcohol or as the acetate, the distortion in the ultraviolet absorption curve is almost entirely in the region below the respective absorption maxima. This distortion accompanies the progressive decrease in the extinction coefficient at 328 millimicrons, though the rate of change is not parallel. During the initial stages of oxidation there is a tendency for the colorimetric values to be somewhat less than those based upon the extinction coefficients at 328 millimicrons, and a reversal of this relationship is observed after prolonged oxidation of the vitamin A. Although the absorption maximum for vitamin A alcohol in isopropanol is closer to 325 than to 328 millimicrons, the difference in extinction coefficient between these two wave lengths is small in the case of most fish liver oils.

The importance of conducting the vitamin A determination on the unsaponifiable fraction has been emphasized. It is possible to prepare unsaponifiable extracts without either mechanical or oxidative loss of vitamin A. The ether used for extracting the saponified oil should be redistilled, since ether, even of U. S. P. quality, contains extraneous light-absorbing substances. Experimental data show that the lower vitamin A values obtained when tests are conducted on the unsaponifiable fractions of even high-potency oils are due not only to the removal of irrelevant absorbing substances but in part to the removal of vitamin A oxidation products in the preparation of the unsaponifiable fractions.

The U. S. P. reference cod liver oil No. 2 is shown to be unsuited as a standard for the spectrophotometric and colorimetric

determination of vitamin A. The ultraviolet absorption curve of this oil does not have the shape characteristic of vitamin A; it contains appreciable quantities of irrelevant light-absorbing material not entirely removed by saponification; it is unstable even when stored under nitrogen in the refrigerator for a period of only one month; and there is a marked discrepancy in its vitamin A content estimated by the spectrophotometric and colorimetric methods on either the whole sample or the unsaponifiable fraction.

Acknowledgment

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Estimation of Vitamin A in Food Products

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The antimony trichloride method for determining vitamin A in food products has been modified to allow corrections for the presence of inhibitors of the color development, for temperature effects, for variations in the reagent, for turbidities produced in the course of the color development, and for extraneous color present in the final test solution. The unreliability of the direct spectrophotometric method for the assay of foods is demonstrated. The reaction of carotene with antimony trichloride has been critically studied.

THE enrichment of many food products with vitamin A (such as margarine, milk, animal feeds, emergency rations, dietary supplements, etc.) has necessitated the development of a suitable control assay procedure. Spectrophotometric methods for determining vitamin A in such products frequently yield erroneous results, even when the measurements are made on unsaponifiable extracts, or when unfortified blank samples are available for the control tests. The modified spectrophotometric procedure involving destructive irradiation of the vitamin A (*5, 12, 14*) to obtain a blank value for irrelevant absorbing materials presumably eliminates the necessity for an unfortified sample. However, this method involves additional analytical steps, the vitamin A is determined by difference, and the blank correction may be very large in relation to the actual vitamin A content.

The Carr-Price colorimetric test for vitamin A (*3*), as modified by Dann and Evelyn (*4*), has been improved to allow corrections for the presence of inhibitors of the color development, for temperature effects, for variations in the reagent, for turbidities produced in the course of the color development, and for extraneous color present in the final test solution. The method has been applied particularly to the determination of preformed vitamin A in food products. The application of the test to fish liver oils (*13*) has proved of value in yielding data to explain various anomalies encountered in spectrophotometric studies.

Reagents

Chloroform, washed three times with an equal volume of water, dried over anhydrous sodium sulfate, distilled, and stored over anhydrous sodium sulfate.

Antimony Trichloride Reagent, prepared by dissolving 125 grams of dry antimony trichloride, c. p., in sufficient chloroform to make a final volume of 500 ml. This solution should be filtered if turbid. It is stable at room temperature for several weeks.

Vitamin A Standard, a chloroform solution containing 100 U. S. P. units of vitamin A per ml. This is prepared by dissolving in chloroform a weighed amount of an oil solution of distilled vitamin A esters (Distillation Products, Inc., Rochester, N. Y.) whose vitamin A content is determined spectrophotometrically using the commercial standard conversion factor of 2000 to convert from $E^{1\%}_{1\,cm.}$ 328 mμ to U. S. P. units of vitamin A. The ultraviolet absorption curve of the distilled esters should duplicate that of vitamin *A* (*13*), and the values obtained for vitamin A on the whole oil and unsaponifiable fraction should agree when determined by both the spectrophotometric and colorimetric methods.

0.5 *N* Alcoholic Potassium Hydroxide, freshly prepared.

0.5 *N* Aqueous Potassium Hydroxide.

Ether, freshly redistilled, discarding the first and last quarters of the distillate.

Apparatus

A direct-reading photoelectric colorimeter, with 620- and 720-millimicron filters. A null-point instrument cannot be used. The direct-reading galvanometer must be critically damped and have a short period. The time required to swing from 0 to 100 and become stable should not be more than 3 seconds. (The Evelyn photoelectric colorimeter, manufactured by the Rubicon Co., Philadelphia, Penna., has proved very satisfactory.)

Interchangeable test tubes for the colorimeter with cork stoppers.

A micropipet, carefully calibrated to deliver 0.1 ml. between two marks.

A 9-ml. automatic pipet (see Figure 1). The opening of the stopcock and the outlet tube should be large enough to permit

complete delivery of the reagent within 1 second. Stopcock lubricants should not be employed.

Analytical Procedure

Preparation of the Unsaponifiable Fraction. A quantity of sample, preferably containing at least 50 U. S. P. units of vitamin A, is saponified by refluxing with the alcoholic potassium hydroxide for 0.5 hour on a water bath. Ten to 15 ml. of alcoholic potassium hydroxide are used for each gram of sample taken. The use of samples larger than 5 grams is not recommended. In the assay of materials low in total solids content it is possible to use a smaller ratio of alcoholic potassium hydroxide to sample. Thus, in the case of enriched milk, 15 ml. of sample are saponified with 45 ml. of the potassium hydroxide solution.

To avoid sampling errors it is desirable, whenever possible, to conduct a preliminary saponification of a large sample, following this by a resaponification of an aliquot using a higher ratio of alcoholic potassium hydroxide to sample. (In margarine, for example, a 10-gram sample is refluxed on a water bath with 45 ml. of alcoholic potassium hydroxide, the suspension is cooled and brought to the 50-ml. mark with the potassium hydroxide solution, and a 20-ml. aliquot is further saponified after the addition of 30 ml. of alcoholic potassium hydroxide.) The solution (or suspension) is allowed to cool, and double its volume of water is added. The resulting solution (or suspension) is then extracted four times with 50 to 100 ml. of ether. The ether extract is washed once with 50 ml. of water, once with 25 ml. of 0.5 *N* aqueous potassium hydroxide, and then with 50-ml. portions of water until the last washing gives no color with phenolphthalein. After drying with anhydrous sodium sulfate it is evaporated to dryness, the last few millimeters being removed at room temperature with a stream of nitrogen. The residue is immediately taken up in sufficient chloroform to give a final concentration of from 5 to 15 U. S. P. units of vitamin A per ml., and the color reaction is then carried out as quickly as possible. A turbid chloroform solution indicates that the ether extract had been insufficiently dried, requiring the addition of anhydrous sodium sulfate to the final test solution.

All operations are performed in amber glassware (*6*).

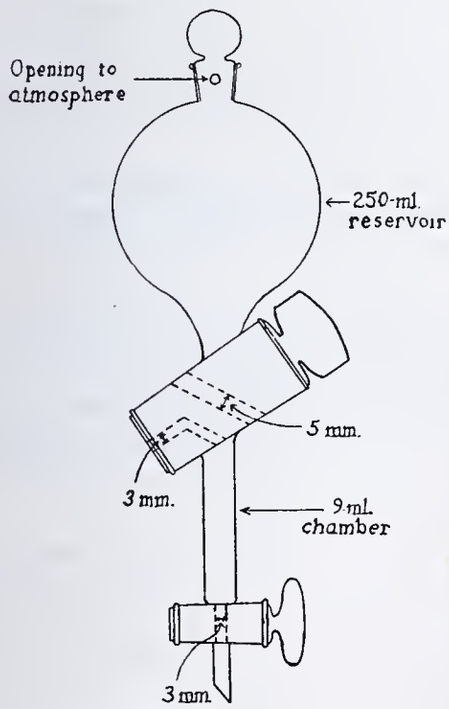

Figure 1. 9-Ml. Automatic Pipet for Delivery of Antimony Trichloride Reagent

Color Development. The center setting of the instrument (using the 620-millimicron filter) is obtained by setting the galvanometer at 100 with 1 ml. of chloroform and 9 ml. of the antimony trichloride reagent. Then to another tube containing 1 ml. of the chloroform test solution are added 9 ml. of the reagent from the automatic pipet. The tube is immediately stoppered and swirled, and the minimal constant galvanometer reading is recorded (*A*) (usually within 4 seconds after the addition of the reagent).

To another tube containing a 1-ml. aliquot of the test solution 0.1 ml. of the vitamin A standard is added from the 0.1-ml. micropipet, followed by 9 ml. of the antimony trichloride reagent, and the minimal constant galvanometer reading is again noted (*B*).

If the test solution is colored, a blank correction is made by measuring the color intensity at 620 millimicrons of a 1 to 10 dilution of the test solution in chloroform (*C*).

To correct for turbidity, occasionally produced during the course of the reaction, the color is developed in a fresh aliquot, and the reading after 4 seconds noted using the 720-millimicron filter (*D*). (The new center setting for the 720-millimicron filter must first be determined as above.)

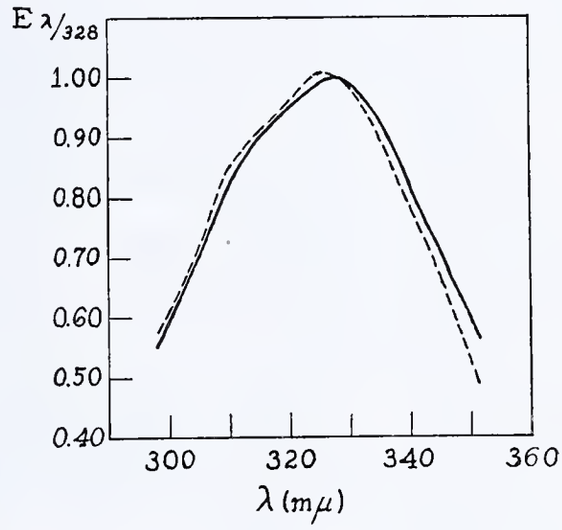

Figure 2. Ultraviolet Absorption Curve of Distilled Vitamin A Esters in Isopropanol

Calculations. Galvanometer readings are converted to photometric densities according to:

$$P.\ D. = 2 - \log G$$

Then,

$$\frac{A - C - D}{1.01\,B - A} \times 10 \times \text{dilution factor} = \text{U. S. P. units of vitamin A per gram of sample}$$

Discussion of Method

Extraneous Color and Turbidity. If the test solution contains pigments that absorb light at 620 millimicrons, the procedure outlined above offers a simple method for correction.

Turbidity, even though not present in the initial test solution, occasionally develops upon the addition of the antimony trichloride reagent. Since the blue color of the vitamin A reaction product shows negligible absorption at 720 millimicrons, the method offers a direct means of measuring and correcting for turbidity. The increment procedure demonstrates that the substances responsible for turbidity do not interfere with the color development.

Selection of the Distilled Vitamin A Esters as the Reference Standard. The ultraviolet absorption curve of this preparation, plotted in Figure 2, is typical of that of vitamin A (see Figure 1 of *13*). At their respective absorption maxima the extinction coefficients of the whole oil and its unsaponifiable fraction show excellent agreement (*13*). The oil solution of the distilled esters is uniform, readily obtainable, and very stable. The extinction coefficient at 328 millimicrons of a stock sample has not changed significantly over a period of 6 months, during which time it was stored under nitrogen in a refrigerator in the absence of light. Even a chloroform solution of the distillate is stable for several days. However, as a precautionary measure, the standard should be freshly prepared each time it is used.

The paper on the spectrophotometric assay of vitamin A (*13*) was concerned in part with experiments on crystalline vitamin A

acetate. This material was selected for that work because it could be considered free of irrelevant absorbing materials and artificial stabilizers. The distilled esters preparation was considered as unsuited since it contains tocopherol. However, in the present study, the presence of tocopherol, imparting greater stability to the oil to be used as the standard, is desirable. The content of tocopherol is insufficient to alter appreciably the absorption curve for vitamin A. Besides, it was suggested by a comparison of the vitamin A values obtained by the spectrophotometric and colorimetric procedures (*13*) that the vitamin A acetate might contain a very small quantity of oxidized vitamin A, insufficient to alter the absorption curve. Thus, if the vitamin A acetate were used as the standard, the colorimetric values for the distilled vitamin A esters preparation and other excellent vitamin A oils would be greater than those derived from spectrophotometric measurements. In the case of the U. S. P. reference cod liver oil No. 2, the discrepancy between spectrophotometric and colorimetric values is extremely large, invalidating its use as a standard for the colorimetric method.

In the method here described, the factor 2000 used for deriving the vitamin A unitage of the standard from the extinction coefficient fixes the colorimetric estimates of potency to this somewhat arbitrary relationship. For practical work with commercial fish liver oils and products with which they are fortified, this is expedient, since trading in oils has been based largely on the spectrophotometric test. The adoption of the commercial standard conversion factor has minimized disputes due to the use of different factors in different laboratories, with insufficient regard for the fact that these factors were no more reliable than the bioassays upon which they were based.

Increment Procedure. The principle of adding to the test solution a known increment of the compound for which assay is being made, rather than basing calculations on a reference curve developed with pure solutions, has been used successfully in several vitamin assay procedures (*1, 8, 10*). By adding a known increment of vitamin A to the chloroform test solution the standard is subjected to the same inhibitory (or accelerating) conditions during the color development as the vitamin A originally present. When graded amounts of vitamin A are added as increments, a linear relationship is observed among them on color development, though the intensity of the colors developed may deviate considerably from those obtained in pure solution. Many naturally occurring chemical inhibitors are not of great concern, since they are largely eliminated by assaying the unsaponifiable fraction (*11*). This can be demonstrated in assaying the U. S. P. reference cod liver oil from freshly opened bottles. On the other hand, such variables as the concentration, water and alcohol content, and stability of the reagent, and the influence of temperature on the reaction can best be compensated for by the increment method.

The use of the distilled esters as an increment in the colorimetric assay of unsaponifiable extracts of foods is justified, since the intensity of the color produced by a given quantity of esterified vitamin A reacting with the antimony trichloride reagent is the same as that for an equivalent weight of the free alcohol (*13*).

Stability of Vitamin A in Chloroform Solution. The distilled vitamin A esters solution dissolved in anhydrous, redistilled chloroform to a vitamin A concentration of 100 U. S. P. units per ml. has been found to be stable for as long as 3 days at room temperature (25° C.). Similar tests on a large series of fish liver oils showed no losses of vitamin A during a 24-hour period. However, an oil is occasionally encountered which shows unusual instability. In such cases the antimony trichloride reaction must be carried out on a freshly prepared chloroform solution.

Comparison of the Colorimetric with the Direct Spectrophotometric Method of Assay. Spectrophotometric vitamin A assays can be regarded as reliable only when an unsaponifiable extract of the substance, dissolved in a suitable solvent, gives an ultraviolet absorption curve typical of vitamin A. This is rarely the case in testing foods, as may be seen in Figures 3 and 4. While in every case there was a slight inflection at 325 millimicrons, where vitamin A alcohol absorbs maximally in isopropanol, the curves are atypical of the vitamin. This indicates that other materials than vitamin A were present which absorbed in this region of the spectrum. Thus, extremely high values for

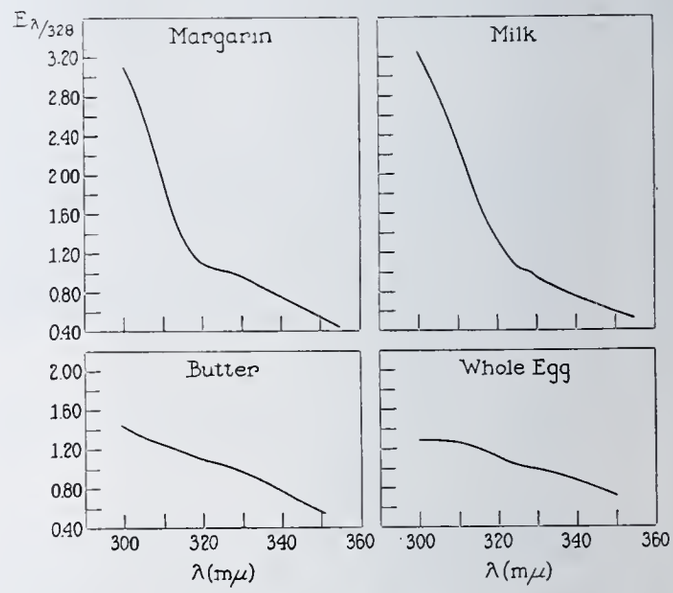

Figure 3. Ultraviolet Absorption Curves of Unsaponifiable Fractions of Margarine, Milk, Butter, and Whole Egg

Table I. Comparison between Spectrophotometric and Antimony Trichloride Tests in Fortified and Natural Foods

Description[a]	Spectrophotometric Test Total	Spectrophotometric Test Blank	Spectrophotometric Test Recovered	Antimony Trichloride Test Total	Antimony Trichloride Test Blank	Antimony Trichloride Test Recovered
	U. S. P. Units of Vitamin A Per Gram					
Fortified margarine, "15,000 units per lb." (33 units per gram)	70.4	38.0	32.4	31.8	0.8	31.0
Fortified milk, "4000 units per quart" (4.06 units per gram added)	12.7	9.13	3.57	4.57	0.65	3.92
Fortified cracker, "1500 units per cracker"[b] (224 units per gram)	156	50	106	116	3	113
Fortified vitamin-mineral soup mix, "5000 units per ounce" (176 units per gram)	430	260	170	190	5	185
Same, after 2 weeks, 42° C.	570	...	...	140	...	...
Same, after 2 weeks, 55° C.	700	...	...	110	...	...
Fortified chocolate malted milk mix, "3000 units per ounce" (106 units per gram)	142	...	...	108	...	...
Fortified vitamin mineral lozenge, "5000 units per wafer" (517 units per gram)	780	...	...	495	...	...
Poultry feed supplement, 200(?) units per gram	400	...	...	195	...	...
Fresh whole egg, 10 to 20 units per gram (*15*)	24	...	...	24 (+6)[c]	...	...
Butter (80% fat), 35 to 50 units per gram (*15*)	33	...	...	18 (+10)[c]	...	...

[a] Values in quotations represent vitamin A added in preparing commercial sample.
[b] Good agreement between spectrophotometric and colorimetric methods, after correction for apparent vitamin A content of unfortified cracker, indicates that expected value was in error.
[c] As carotene determined by chromatographic adsorption.

Table II. Reliability of Method for Determining Carotene by the Antimony Trichloride Reaction

Test Solution (in 1 ml. of $CHCl_3$)	Photometric Density, 620 Millimicrons: 4 seconds	1.0 minutes	30 minutes	2 hours	3 hours	Comment
10 U. S. P. units of vitamin A (approximately 3 μg.)	0.270	0.226	0.025	0.016	0.016	Typical vitamin A reaction
60 μg. of β-carotene	0.222	0.209	0.268	0.321	0.328	Typical carotene reaction
10 U. S. P. units of vitamin A + 60 μg. of β-carotene	0.502	0.447	0.297	0.323	0.323	Carotene and vitamin A reactions additive
Conclusion: Antimony trichloride reaction can differentiate quantitatively between vitamin A and carotene in pure solutions.						
						"Carotene", μg./g.
Unsaponifiable extract of 0.26 gram of whole egg	0.276	0.295	0.166	0.161	0.173	By $SbCl_3$ reaction = 126
Same + 10 U. S. P. units of vitamin A	0.536	0.517	0.188	0.161	0.167	By A. O. A. C. method (2) = 5.0; By chromatographic adsorption (7) Total = 4.5
Same + 60 μg. of β-carotene	0.464	0.506	0.420	0.456	0.468	β-carotene = 2.5
Unsaponifiable extract of 0.38 gram of butter	0.202	0.170	0.042	0.041	0.043	By $SbCl_3$ reaction = 22
Same + 10 U. S. P. units of vitamin A	0.457	0.376	0.059	0.054	0.056	By A. O. A. C. method (2) = 7.1; By chromatographic adsorption (7) Total = 6.4
Same + 60 μg. of β-carotene	0.447	0.403	0.300	0.329	0.332	β-carotene = 5.8
Conclusion: Antimony trichloride reaction on unsaponifiable fraction of above products measures other compounds in addition to carotene and vitamin A.						

vitamin A are obtained when the estimates are based on a single spectrophotometric reading at 328 (or 325) millimicrons, even if unsaponifiable extracts are tested.

In the case of fortified preparations the difference between the extinction coefficients at 328 millimicrons of the vitamin A enriched sample and an unfortified, though otherwise identical, blank sample may be used for calculating the amount of vitamin A added. However, this is a difference method, necessitating two separate analyses and the use of a blank sample, which is not always available. Furthermore, the blank value is often very high relative to the total absorption at 328 millimicrons, thus decreasing the precision of the method. In the case of some blank samples there are present appreciable quantities of vitamin A (as, for example, in milk), and while a fairly good approximation of the vitamin A added in fortification may be obtained, a reliable estimate of the total vitamin content of the enriched sample cannot be made by this spectrophotometric procedure.

In Table I are presented the results of assays using both the spectrophotometric and colorimetric methods. In each case confirmatory data were obtained by assays conducted on several preparations in each category. The spectrophotometric blank values obtained for the samples tested were high. On the other hand, in the antimony trichloride tests, the blank values were so low that they could actually have been ignored. Only in the case of milk was this figure appreciable, accounting for approximately 14 per cent of the vitamin A present in the fortified product. This value, however, actually represented in large part vitamin A and is really not a blank in the usual sense of the word.

The unreliability of the spectrophotometric method for estimating the vitamin A content of food materials is clearly demonstrated by the values obtained for a sample of a vitamin- and mineral-fortified soup mix. While the apparent vitamin A content as determined spectrophotometrically increased on storage at an elevated temperature, owing to the formation of nonspecific absorbing substances, the antimony trichloride test showed a decrease in vitamin A content, as was expected. Unfortunately, no stored blank samples were available, but if they had been the spectrophotometric vitamin A values of the incubated samples might have agreed better with the colorimetric figures.

In the case of those samples, which naturally contain inappreciable amounts of vitamin A, the modified spectrophotometric method, involving destructive irradiation of the vitamin A, should theoretically yield the same blank values as those recorded by the direct method for the unfortified samples. Thus, the application of this spectrophotometric technique to the assay of enriched food products, such as those in Table I, must sacrifice precision for specificity. In addition, in any such method it must be shown that the vitamin A is quantitatively converted by the irradiation to materials which fail to absorb light at 328 millimicrons and that the extinction coefficients at 328 millimicrons of substances other than vitamin A are not appreciably affected by this treatment.

For the two natural products listed in Table I—namely, whole egg and butter—the direct spectrophotometric values for preformed vitamin A were not so unreasonable when compared with the bioassay figures for total vitamin A reported in the literature (*15*). However, the ultraviolet absorption curves of the unsaponifiable fractions of these products demonstrated that substances other than vitamin A were present, absorbing light at 325 millimicrons. Thus, the spectrophotometric values must be regarded as being erroneously high. This also casts suspicion on the reliability of the colorimetric value, particularly for whole egg.

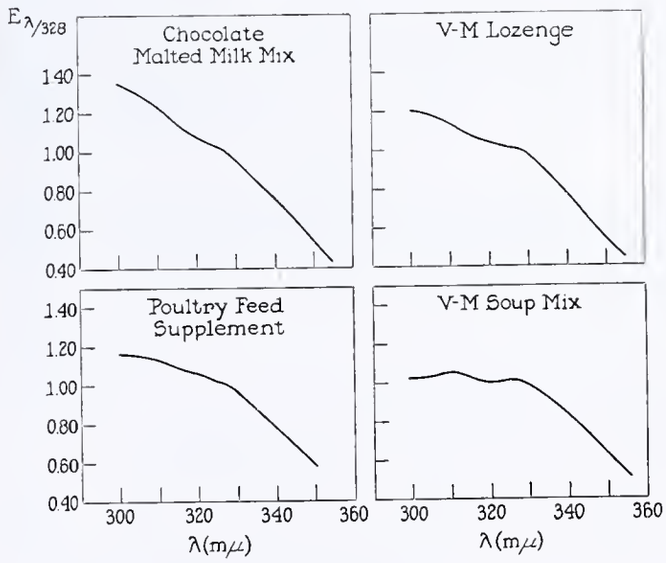

Figure 4. Ultraviolet Absorption Curves of Unsaponifiable Fractions of Chocolate Malted Milk Mix, Vitamin and Mineral Lozenge, Poultry Feed Supplement, and Fortified Soup Mix

The reliability of the spectrophotometric determination of vitamin A in milk was not improved when the saponification was conducted on an ether extract of the milk.

Determination of Preformed Vitamin A and Carotene in the Presence of Each Other. The antimony trichloride test, as applied in the present study, is intended for the measurement of only preformed vitamin A. Carotene also yields a blue color with antimony trichloride (*9*), though of much lower intensity on a weight for weight basis. Furthermore, the color

reaction with carotene differs from the vitamin A reaction in that it attains its maximal intensity only after standing, instead of fading from an almost instantaneous maximum. In Table II the difference between these two reactions is shown.

The maximal color produced by 10 U. S. P. units (about 3 micrograms) of vitamin A and measured at 620 millimicrons is of approximately the same order as that produced by 60 micrograms of β-carotene, although the time at which these maximal intensities are reached differed. Thus, on the basis of equal weights, the ratio of the color intensity of the reaction product of preformed vitamin A to that of β-carotene is approximately 20 to 1; on the basis of biological activity, it is 10 to 1. These data also show that vitamin A and carotene, in solution together, react in an additive manner. In the case illustrated the photometric density at any given time closely approximates the sum of the two individual values. After an interval of 2 hours, when the blue color due to reacted vitamin A has almost completely disappeared, the residual photometric density is a measure of the β-carotene present. Thus, the antimony trichloride reaction can be used to differentiate quantitatively between vitamin A and β-carotene in pure solutions, according to the following equations:

$$(P.\ D.\ \text{at 4 seconds}) - \frac{0.222}{0.321} \times (P.\ D.\ \text{at 2 hours}) = P.\ D.\ \text{due to preformed vitamin A}$$

and

$$(P.\ D.\ \text{at 2 hours}) = P.\ D.\ \text{due to } \beta\text{-carotene}$$

where

$$P.\ D. = \text{photometric density} = 2 - \log G$$

Attempts were made (see Table II) to apply this method to such natural products as whole egg and butter, which contain appreciable quantities of both preformed vitamin A and carotene. In the case of the whole egg the photometric density of the test solution reached a maximum in 4 seconds, diminished promptly, and after about 0.5 hour reached a level at which it remained constant. A sufficient time was then allowed for the disappearance of the color of the vitamin A reaction product. Fully 2 hours were required, as indicated by the fact that only after this period did the photometric density of the solution, containing the known increment of vitamin A, equal that of the test solution.

The photometric density after 2 hours was then attributed to reacted carotene. The test was repeated after adding 60 micrograms of β-carotene to another portion of the original test solution, and the photometric density after 2 hours again recorded. Using this increment in photometric density as the standard, the β-carotene content of the unfortified sample was computed to be 126 micrograms of β-carotene per gram. This value is outside the range of probability, since the simple A. O. A. C. (*2*) colorimetric method for crude carotene gave a value of 5 micrograms per gram, and the more specific chromatographic adsorption procedure (*7*) showed 4.5 micrograms. In other words, it appears that in whole egg, compounds other than β-carotene are present which give a stable antimony trichloride reaction product absorbing light at 620 millimicrons. (In egg the xanthophylls comprise a large fraction of the carotenoid pigments.) The presence of preformed vitamin A in the unsaponifiable extract prepared from butter was demonstrated by the fact that the initial photometric density was maximal, and promptly decreased to a minimum in 30 minutes, after which it remained constant. However, the estimate of the carotene content from the residual stable photometric density value of the test sample and that produced by a known increment of β-carotene is likewise erroneous; the estimate of 22 micrograms per gram of β-carotene is considerably higher than the value found by the A. O. A. C. colorimetric procedure or by chromatographic adsorption. It is apparent, therefore, that the antimony trichloride method, as described, cannot be used for the determination of carotene in foods.

The use of this reaction for the determination of preformed vitamin A may be subjected to some criticism, since it is assumed that the stable readings obtained after 2 hours are due to compounds which, although not β-carotene, behave like it. When the 2-hour reading is large relative to that noted at the end of 4 seconds (58 per cent in the case of the whole egg), errors due to the above assumption may be appreciable, and may therefore invalidate the test. When the stable reading is small relative to that at 4 seconds (20 per cent in the case of butter), a more reliable value for preformed vitamin A is obtained.

In the authors' opinion, preformed vitamin A in food materials should be estimated by the improved antimony trichloride reaction and carotene should be estimated independently, preferably by chromatographic adsorption. A small correction may then be applied for the reaction of the carotenoids with the antimony trichloride reagent in the test for vitamin A. It is apparent, from the results shown in Table II, that in testing a product in which as much as half of the vitamin A potency is due to β-carotene, the amount of color at 4 seconds due to the reaction of carotene is small, of the order of 8 per cent.

The simple procedure, suggested by Dann and Evelyn (*4*), of measuring the yellow color of the chloroform solution prior to its reaction with the antimony trichloride reagent and attributing this to carotene, has been found to yield "carotene" values for a number of food materials as much as six times greater than those obtained by the simple A. O. A. C. (*2*) colorimetric procedure, which is admitted to be not specific for the biologically active carotenoids (*7*).

Summary

A modification of the antimony trichloride method is described for determining vitamin A in food products. The method compensates for the effect of inhibitors of the color reaction, variations in the reagent, temperature effects, and extraneous colors and turbidity that sometimes interfere.

When foods are assayed by the spectrophotometric procedure, erroneously high estimates are usually obtained despite preliminary extraction of their unsaponifiable fractions for the tests. In such cases the absorption curves deviate markedly from the characteristic vitamin A curve. The use of unfortified blank samples (if they are available) increases the specificity of this method with the possible sacrifice of precision. The modified spectrophotometric procedure involving ultraviolet destruction of vitamin A is a "difference method", the blank corrections being large relative to the true vitamin A values. In the colorimetric method a blank sample is usually unnecessary because of the greater specificity of the procedure for determining vitamin A in foods.

β-Carotene reacts with the antimony trichloride reagent to give a stable blue color. Determination of both carotene and preformed vitamin A, in the presence of each other in pure solutions, is possible by this reaction. However, in natural foods, substances other than carotene yield blue reaction products, invalidating use of this test for the determination of carotene. The amount of color due to the reaction of pure β-carotene with the reagent is very much less than that due to preformed vitamin A, expressed either on the basis of equal weight or equivalent biological activity. Thus, only a minimal correction for β-carotene, if any at all, need be applied to colorimetric preformed vitamin A values in the assay of foods containing both forms of the vitamin. Carotene should be estimated independently, preferably by chromatographic adsorption.

Acknowledgment

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Polarographic Analysis of Dilute Solutions of Bismuth

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Data are presented for the quantitative polarographic analysis of dilute solutions of bismuth in the form of the complex tartrate in acetate-buffered solutions. Such solutions (10^{-5} to 3×10^{-3} molal) can be analyzed with an average error of 1 mg. of bismuth per liter. The diffusion current is not quite a linear function of the bismuth concentration from 1×10^{-5} to 3×10^{-3} molar solutions at the pH value and tartrate concentration investigated in this work. The half-wave potential is a function of the pH of the solution.

In the course of an investigation of the ionic equilibria of bismuth it became necessary to analyze dilute solutions of bismuth (in the range 10^{-2} to 10^{-5} molal). This paper presents data on the use of the polarographic method for the analysis of such solutions.

Early attempts were made to obtain polarograms for the reduction of bismuth in hydrochloric acid using a Heyrovský micropolarograph[2]. At these high hydrogen-ion concentrations the half-wave potential was so close to zero applied potential that little or no part of the residual current curve was obtained to be used in evaluating the diffusion currents. In addition, the curves exhibited erratic maxima. However, the half-wave potential of a bismuth-tartrate complex ion is known to be more negative than that of Bi^{+++} by several tenths of a volt (*6*). Such a system gave promise of avoiding the difficulty experienced with the hydrochloric acid solutions; a brief study of this system is reported in this paper.

Apparatus

A manual apparatus, similar to that of Lingane and Kolthoff (*3*), was used for this work. A 9999-ohm decade resistance box was connected in series with the cell consisting of the dropping mercury electrode and a normal calomel half-cell. The drop in potential across this resistance was measured with a student potentiometer (±0.0001 volt). The currents flowing were calculated from Ohm's law. Another student potentiometer was used as a volt-box for tapping off known potentials (to be impressed across the cell) from Edison cells. A galvanometer (2×10^{-8} ampere per mm. sensitivity) was used for null-point readings only. The dropping mercury electrode was attached to a leveling bulb and manometer (*3*). No rubber was in contact with the mercury. With a head of 35.0 cm. of mercury, an average of 1.443 mg. of mercury flowed from the capillary each second when immersed in the solution used and at a potential of 0.500 volt across the cell. The drop time averaged 3.43_5 seconds, giving the value of 1.57 for the product $m^{2/3}t^{1/6}$ (*2*). All work was done with the cells thermostated at 35° ± 0.05° C.

Materials

Grasselli's c. p. hydrochloric acid, Baker's analyzed (reagent quality) bismuth chloride, glacial acetic acid, sodium hydroxide, and sodium acetate, and Coleman-Bell's c. p. tartaric acid were used throughout this work. All solutions were made with double-distilled water.

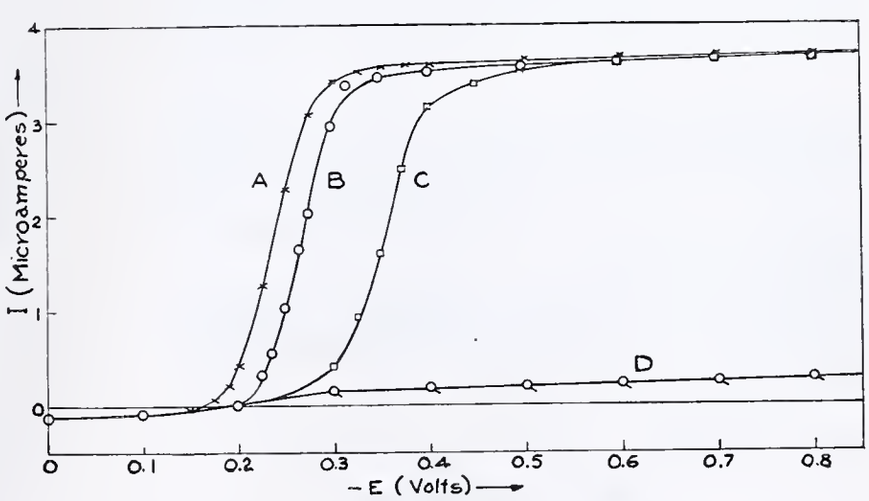

Figure 1. Typical Polarograms

A. 100 mg. of bismuth per liter, pH 4.20
B. 100 mg. of bismuth per liter, pH 4.75
C. 100 mg. of bismuth per liter, pH 5.90
D. 0 mg. of bismuth per liter, pH 4.75
Each solution was 0.1 molar in tartrate and buffered with acetic acid–sodium acetate mixtures

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[2] Page and Robinson (*4*) have recently published an account of the polarographic analysis of bismuth in hydrochloric and sulfuric acid solutions. Most of the authors' work was done before this paper was published.

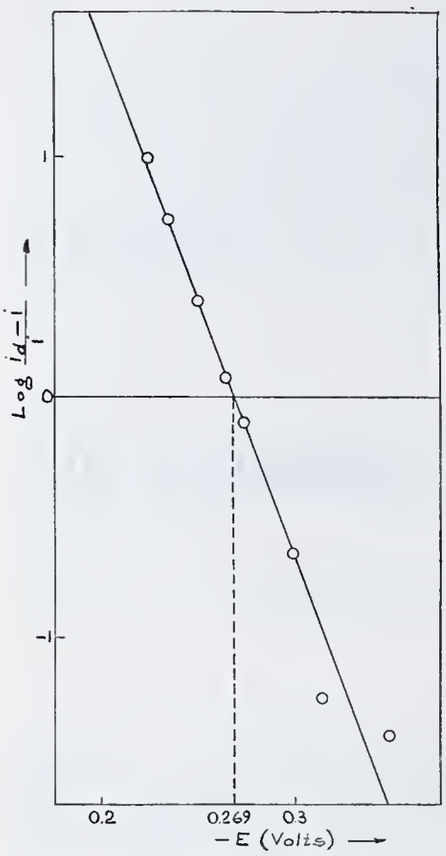

FIGURE 2. PLOT USED TO FIND VALUE OF HALF-WAVE POTENTIAL OF *B*, FIGURE 1

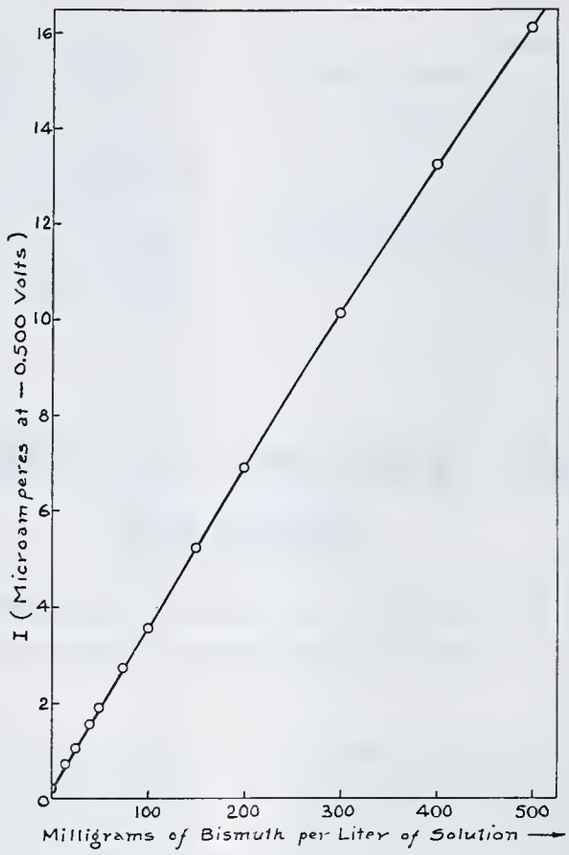

FIGURE 3. VARIATION OF MEASURED CURRENT WITH CHANGE OF BISMUTH CONCENTRATION

All solutions were 0.1 molar in tartrate, 0.05 molar in acetic acid, and 0.05 molar in sodium chloride. pH 4.75 ± 0.05

solutions containing the supporting electrolyte only—i. e., tartrate and acetate buffer system (*cf.* Figure 1, *D*)—from the measured currents.

The half-wave potentials were obtained from a plot of E *vs.* $\log \frac{i_d - i}{i}$ where $E = E_{1/2}$ when $\log \frac{i_d - i}{i} = 0$. This plot for curve *B*, Figure 1, is shown in Figure 2.

Currents (measured as total current at −0.500 volt) at various bismuth concentrations are shown graphically in Figure 3. The curve is strictly linear below a bismuth concentration of 100 mg. per liter. The ratio of i_d (microamperes) to concentration of bismuth (mg. per liter) has an average value of 0.0340 in this range. Above a bismuth concentration of 100 mg. per liter the curve deviates slightly from linearity. At a bismuth

Experimental

A standard solution of bismuth trichloride in hydrochloric acid was prepared. It was standardized gravimetrically by removing the chloride ions by twice evaporating the solution nearly to dryness with excess nitric acid, precipitating the bismuth as the basic carbonate, and igniting the basic carbonate to the oxide which was weighed. All other solutions of bismuth were prepared by dilution of this standard solution in calibrated flasks.

Atmospheric oxygen was removed from all samples by bubbling purified nitrogen through them for 10 minutes.

Dilute bismuth solutions, 0.1 molar in tartrate and buffered with acetic acid–sodium acetate mixtures, were investigated; they showed well formed polarographic waves. At high bismuth concentrations the waves exhibited small maxima but these were completely eliminated by the addition of one drop of 0.1 per cent potassium methyl red solution per 25 cc. of solution. The results are shown in Figures 1 to 4.

The relation between the diffusion current and the concentration of bismuth (present as tartrate complex) was determined by obtaining polarograms on solutions which varied in bismuth concentration but all of which were 0.1 molar in tartrate, 0.05 molar in acetic acid, and 0.05 molar in sodium acetate. All these solutions had a pH of 4.75 ± 0.05. All diffusion currents were measured at a potential of −0.500 volt with reference to the normal calomel electrode.

Suchý (*6*) reported a half-wave potential of −0.34 volt for this reduction, but all the authors' work on the acetate-buffered bismuth tartrate solutions showed values of about −0.27 volt. To account for this discrepancy, the effect of change of pH on the half-wave potential was investigated.

The pH determinations were carried out with a glass electrode using a 0.0500 molar potassium acid phthalate solution as a standard (pH = 3.97, *1*).

Data and Discussion

Typical data for complete polarograms of the bismuth-tartrate complex are shown graphically in Figure 1 where I is the measured current in microamperes.

Diffusion currents, i, and limiting diffusion currents, i_d, were obtained by subtracting the residual currents, obtained with

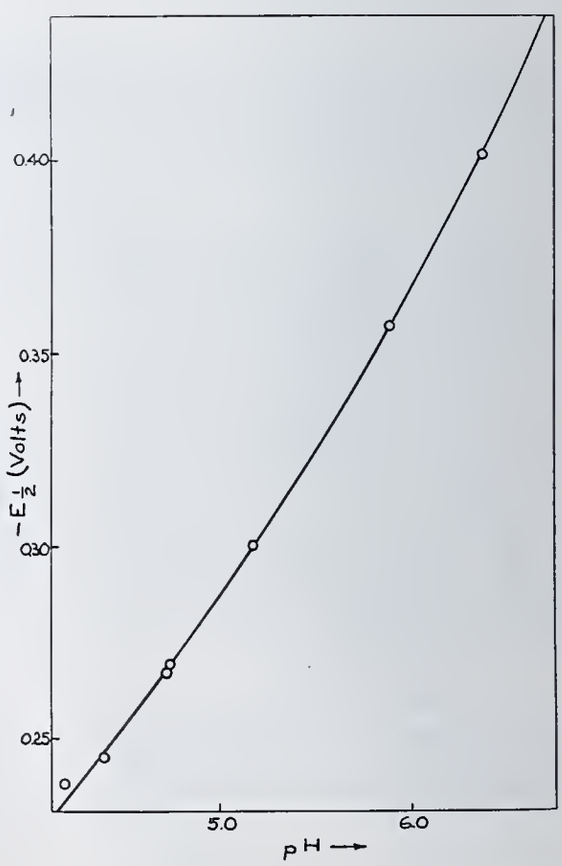

FIGURE 4. HALF-WAVE POTENTIAL AS A FUNCTION OF pH

For solutions containing 100 mg. of bismuth and 0.1 mole of tartrate per liter

concentration of 300 mg. per liter the above ratio drops to 0.0330, at 500 mg. per liter to 0.0318, and at 600 mg. per liter to 0.0308.

It was found that high concentrations of sodium chloride lowered the slope of the curve in Figure 3 proportionately to the sodium chloride concentration. One molar sodium chloride lowered the slope from 0.0340 to 0.0318, and 2 and 3 molar sodium chloride lowered it to 0.0296 and 0.0276, respectively. This effect has been noted by Peracchio and Meloche (*5*).

The polarographic data, plotted on a large scale, were used for the analysis of dilute solutions of bismuth. An extrapolation of this plot (Figure 3) to zero concentration gave an accurate value of the residual current of 0.20 microampere at −0.500 volt. Values of the diffusion currents were reproducible to an equivalent of about 1 mg. of bismuth per liter at all concentrations.

The different values of $E_{1/2}$ (reported by Suchý, *6*, and that above) are explained by the fact that $E_{1/2}$ is shifted greatly by change of pH of the solutions. Figure 4 shows a plot of $E_{1/2}$ versus pH for solutions having 100 mg. of bismuth per liter. The value of the diffusion current is not affected by change of pH, if pH < 7, within the limits of experimental error. Changes of pH below 7 only translate the wave along the E axis, Figure 1. For pH > 7 the current voltage curves showed two waves suggesting a stepwise reduction of bismuth but no definite mechanism is proposed to interpret these two waves.

The shift of $E_{1/2}$ to greater negative potentials as the pH values increase corresponds to the well known fact that metal-tartrate complex ions in general are more stable in alkaline than in acid media.

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A Modified Bailey Pressure Regulator

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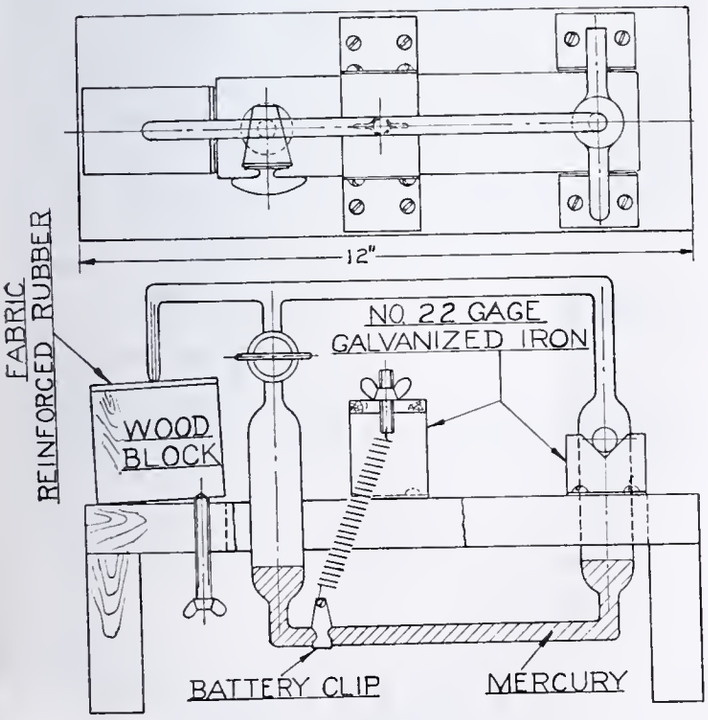
BAILEY (*1*) recently described a manostat which, under certain circumstances, was inclined to "bounce", and explained how the bouncing could be eliminated by grinding a bevel on the tip of the capillary leak.

A duplicate was constructed by the author, but it was found that the bevel caused a poor seal between the tip and the rubber seat and prevented attaining a vacuum lower than about 40 mm.



MANOSTAT

in the apparatus. Furthermore, bouncing set in when attempt was made to set the pressure at 80 mm. Several tips were constructed with various bevels and tried with rubber seats of various hardnesses. As was to be expected, increasing the inside diameter of the tip and the angle of the bevel increased the average pressure at which the manostat functioned smoothly. Each tip covered a range of about 30 mm.

The modification shown in the diagram is, in effect, a variable bevel. The capillary is first drawn down to a very fine tip and then ground down on a steel plate, using coarse emery and kerosene, until the opening is of such a size that when unobstructed and connected into the apparatus, the pump will take the pressure down to a value which is about 20 mm. higher than the highest pressure required during distillation.

To adjust for operation, mercury is placed in the manostat, the block is removed, the stopcock is opened, and the spring tension is adjusted so that the tip will come to rest about 1 cm. below the level of the top of the block.

The block is placed in position, the pump is started, and the tip is pushed firmly by hand into the rubber. When the pressure as indicated on a manometer reaches its lowest value, the hand is removed and the manometer will show the lowest pressure the manostat can maintain.

The setscrew is raised, increasing the leak at the tip until the pressure in the system is about 10 mm. less than the pressure desired. The tip is then lifted free of the seat with one hand and the stopcock is flipped over with the other. This procedure permits air to enter the chamber over the mercury, and increases the pressure for which the manostat is set. The setting can be decreased by pushing the tip against the seat and flipping the stopcock.

With a little practice, the manostat can thus be set within 2 to 3 mm. of the desired pressure. The final adjustment is made with the setscrew and an accuracy of 0.5 mm. in the setting is easily attained. Any drift due to fatigue of the rubber or dirt particles can be quickly neutralized by means of the setscrew.

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Spectrographic Determination of Nickel and Chromium in Stainless Steel

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Nickel and chromium in stainless steel can be determined by the spectrographic method, using the interrupted spark as a source of radiation. The method is derived from the conventional "internal standard" procedure, and does not require the presence of a "standard" element in a fixed percentage. Details of procedure for preparing working curves and making determinations are given. The method is recommended for control of routine melting operations. Its precision compares satisfactorily with ordinary analytical determinations.

THE internal standard method of spectrographic analysis as commonly used is dependent upon the assumption that the concentration of the element used as a standard does not vary from one sample to another. It is frequently desirable to analyze materials consisting of three component elements present in various percentages. A typical material of this type is stainless steel in which the principal constituents are nickel, chromium, and iron. Typical analyses show that elements present in minor amounts, such as silicon, carbon, manganese, sulfur, etc., will constitute approximately 2 per cent of the material, as manufactured under average conditions. Therefore, the relation of the principal components of a stainless steel may be represented by the equation

$$\%\,\mathrm{Ni} + \%\,\mathrm{Cr} + \%\,\mathrm{Fe} = 98 \qquad (1)$$

The ratio of the concentration of nickel to the concentration of chromium may be represented by X, and the ratio of the concentration of iron to the concentration of chromium may be designated by Y. Then if quantities X and Y can be experimentally determined, the concentrations of the elements can be determined by the solution of the algebraic equations:

$$\begin{aligned} \mathrm{Ni} + \mathrm{Fe} + \mathrm{Cr} &= 98 && (1)\\ \mathrm{Ni} - X\mathrm{Cr} &= 0 && (2)\\ \mathrm{Fe} - Y\mathrm{Cr} &= 0 && (3)\\ \hline \mathrm{Cr} &= \frac{98}{1 + X + Y} && (4)\\ \text{and} \qquad \mathrm{Ni} &= X\mathrm{Cr} && (2) \end{aligned}$$

A spectrographic method for determining concentration ratios X and Y has been developed and applied with satisfactory results for the control of the melting of stainless steel in the electric furnace.

A series of five samples of stainless steel was prepared by melting in an induction furnace and casting small rods in a cast-iron mold. The rods were 5 cm. (2 inches) long and 0.6 cm. (0.25 inch) in diameter. Casting in the iron mold caused very rapid freezing of the sample, so that segregation at the grain boundaries was reduced to a minimum. The rods were ground so that the ends were conical, having an included angle of 140°. They were aligned axially in electrode holders to form a 2-mm. spark gap.

The excitation energy was supplied by a commercial type (A. R. L., Dietert) high-voltage spark circuit including a synchronous interrupter. The circuit was adjusted to contain inductance of 0.04 millihenry and capacitance of 0.007 microfarad. The power input was $^2/_3$ kva. According to the manufacturer, the peak voltage across the condenser is 35,000 volts. The sample was sparked for 30 seconds before beginning the exposure. The presparking time and the sparking conditions must be accurately duplicated in order to obtain satisfactory reproducibility.

The spectrograph used was a Baird (*1*) 3-meter grating instrument, and the spectrum was photographed in the second order. The exposure time for Eastman Spectrum Analysis No. 1 film was of the order of 10 seconds. The film processing was performed in an accurately reproducible manner (*4*) in solutions held at a thermostatically controlled temperature of 70° F.

The series of five samples of stainless steel was carefully analyzed by chemical methods (Table I). The spectra of these five samples were studied and three lines were chosen for measurement. The lines used for the measurements described in this paper are listed in Table II with the data given in the M. I. T. Wavelength Tables (*2*). Later work has shown that these lines are subject to interference when copper, molybdenum, or titanium is present; hence a more suitable group of lines is Ni 3087.077, Cr 3169.192, and Fe 3259.048.

Since the densitometer used in this laboratory indicates percentage transmission, it is convenient to prepare calibration curves in terms of this quantity.

TABLE I. CHEMICAL ANALYSIS OF STANDARD SAMPLES

Sample No.	1	2	3	4	5
Nickel, %	7.72	14.1	17.5	26.2	35.7
Chromium, %	20.2	28.6	28.5	17.6	19.4
X = Ni/Cr	0.382	0.493	0.614	1.49	1.84
Y = Fe/Cr	3.42	1.90	1.79	3.04	2.06

TABLE II. SPECTRUM LINES SELECTED FOR MEASUREMENT

Wave Length	Element	Intensity Arc	Intensity Spark
2997.301	Fe II	...	60
3087.077	Ni II	...	150
3111.941	Cr II	...	40

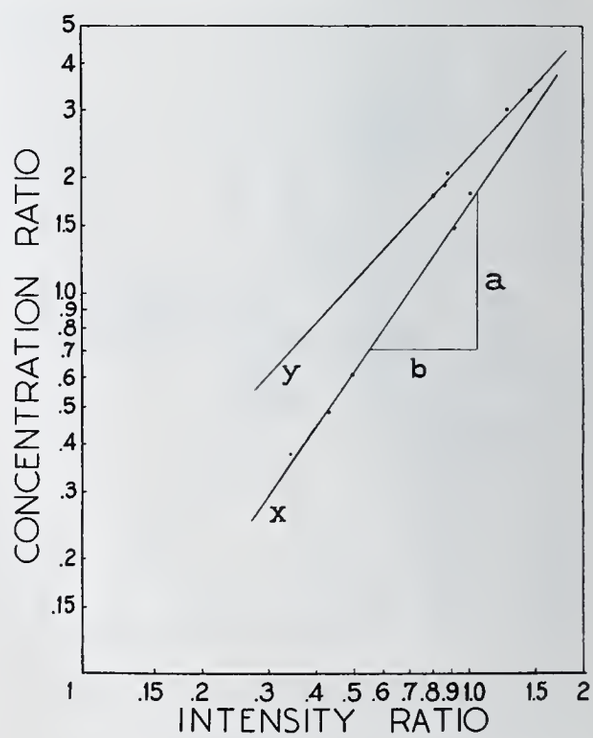

FIGURE 1

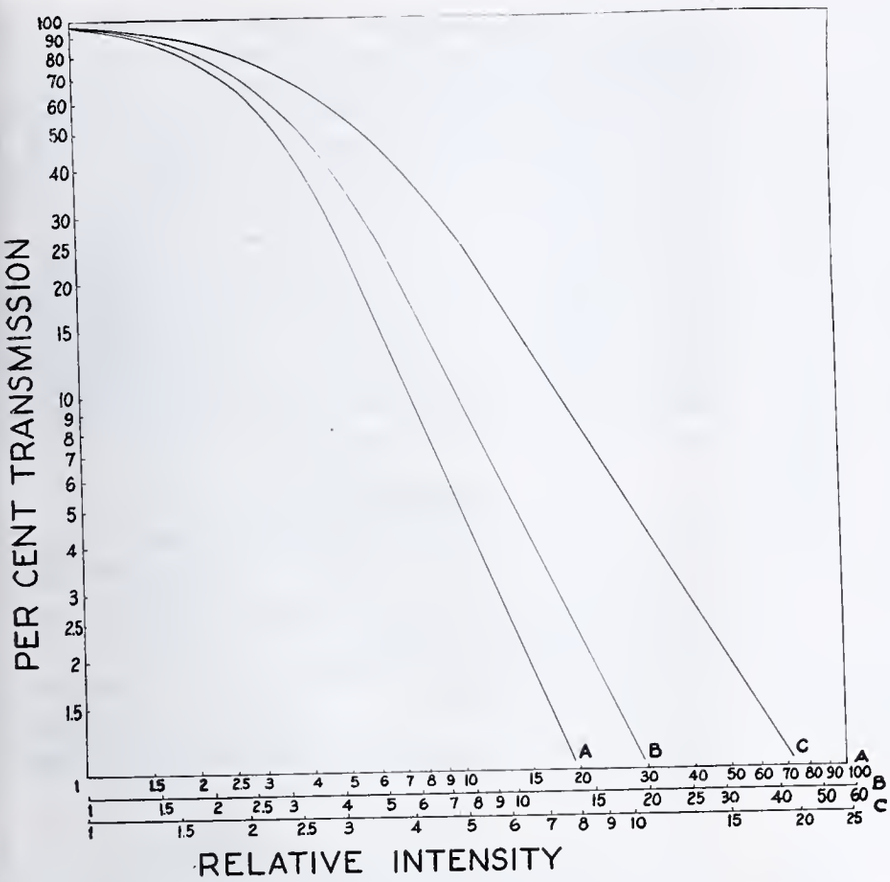

FIGURE 2

The log T/log I curve (Figure 2, A), for the film emulsion was determined by using a step sector, and was plotted on a calculating board as suggested by King (*3*). The spectra of the five samples were measured and the intensity ratios of the lines Ni 3087/Cr 3111 and Fe 2997/Cr 3111 for each sample were determined from the log T/log I curve. The logarithms of the intensity ratios were plotted against the logarithms of concentration ratios X and Y (Figure 1).

The slopes (a/b) of the curves in Figure 1 were determined. A working curve for the value of X (Figure 2, C) was prepared by plotting the data of the log T/log I curve on the same vertical scale and an expanded horizontal scale (scale C, Figure 2). The expanded scale was obtained by magnifying scale A by the ratio a/b determined from curve X of Figure 1. A modified ratio of the intensities of the lines Ni 3087/Cr 3111 for a standard sample was read from this working curve. The Ni/Cr index constant was determined by dividing the Ni/Cr concentration ratio of the standard sample by the Ni 3087/Cr 3111 modified intensity ratio. A working curve for the value of Y (Figure 2, B) was prepared, and the Fe/Cr index constant was determined in similar manner. The expanded logarithmic scales (B and C, Figure 2) can be prepared conveniently for use on the calculating board by photographic enlargement.

The calculating board is used for determining the value of X for a given sample as follows:

1. Set the vertical scale of the calculating board so that it intersects curve C of Figure 2 at the per cent transmission of Cr 3111.
2. Set the value of the Ni/Cr index constant on horizontal scale A to coincide with the index line.
3. Move the vertical scale so that it intersects curve C at the per cent transmission of Ni 3087.
4. Read the value of X on scale A under the index line.

Y is determined in the same manner, using curve B of Figure 2, the transmissions of Cr 3111 and Fe 2997 with the Fe/Cr index constant.

In order to obtain the greatest accuracy a complete set of curves should be prepared for each plate or film, but this is not feasible in high-speed routine work. Since the log T/log I curve is determined by the characteristics of the emulsion and the developing procedure, the use of accurate time and temperature control makes it necessary to redetermine this curve only when a different emulsion or a new batch of developer is put in service.

The concentration ratio *vs.* intensity ratio curves are determined by the type of material, the sample, and the excitation procedure. Hence, it should be necessary to redetermine these curves only when changes are made in one or more of these factors. The desirability of exact repetition of the excitation procedure with particular regard to voltage stabilization and timing should be emphasized.

The routine of analysis consists of five steps: (1) preparation of sample, (2) exposure and processing of spectrogram, (3) measuring per cent transmissions of three spectral lines, (4) reading concentration ratios X and Y from the working curves, and (5) slide-rule computation of the concentrations from Equations 2 and 4.

In order to study the reliability of the method in routine analysis, a number of heats were analyzed by both chemical and spectrographic methods (Table III). The agreement between chemical and spectrographic values is indicated by expressing the spectrographic value as a fraction of the chemical value. The standard deviations of the two series of ratios are shown in Table IV. The per cent deviations in Table IV are due to the combined errors of the chemical and spectrographic procedures; all were routine determinations in which no special effort was made to attain high accuracy.

TABLE III. COMPARISON OF ROUTINE CHEMICAL AND SPECTROGRAPHIC DETERMINATIONS

	Chromium			Nickel		
Heat No.	Spectrographic	Chemical	Ratio of spectrographic to chemical	Spectrographic	Chemical	Ratio of spectrographic to chemical
2170	29.89	28.70	1.042	15.37	15.42	0.997
2173	29.62	26.48	1.118	15.72	16.20	0.970
2190	30.68	27.97	1.096	15.63	16.08	0.972
2193	29.78	28.56	1.033	15.78	15.87	0.994
2197	29.30	27.78	1.055	15.56	15.72	0.990
2703	28.85	28.97	0.996	14.82	15.17	0.977
2725	28.70	28.12	1.020	15.23	14.38	1.059
2728	28.91	27.99	1.033	15.02	15.80	0.951
2731	30.52	29.18	0.955	15.00	15.80	0.949
2740	30.00	29.46	1.018	14.58	15.09	0.966
2743	28.92	28.23	1.024	15.18	15.01	1.011
2746	31.56	29.17	1.083	14.92	14.66	1.018
2750	28.50	27.21	1.047	14.81	14.50	1.021
2792	30.80	29.31	1.050	15.40	15.16	1.016
2795	32.00	30.82	1.037	16.32	16.28	1.002
2797	32.21	31.67	1.017	15.75	16.50	0.955
2800	29.79	30.14	0.988	16.37	14.47	1.131
2816	30.37	29.60	1.026	15.06	14.38	1.047
2819	29.59	30.47	0.972	14.26	15.41	0.925
2821	31.12	30.23	1.029	16.10	15.41	1.045
2822	30.31	28.84	1.051	14.91	16.44	0.907
2825	28.74	28.40	1.012	20.69	20.23	1.023
2827	30.57	29.12	1.048	20.65	20.23	1.021
2829	29.75	28.57	1.042	20.90	20.90	1.000
2832	30.80	29.20	1.054	20.95	20.90	1.002
2835	28.13	29.72	0.947	19.19	19.48	0.985
2844	27.72	27.39	1.013	24.53	23.50	1.044
2847	28.88	28.01	1.031	20.07	20.03	1.002
2851	30.35	30.14	1.007	20.55	19.96	1.030
2854	30.77	29.38	1.047	21.08	20.44	1.031
2865	30.21	29.23	1.033	21.05	21.00	1.002
2868	28.47	27.90	1.019	24.08	22.62	1.065
2871	29.75	28.26	1.052	20.97	21.00	0.999
2874	30.63	29.20	1.049	20.97	21.20	0.989
2884	31.65	29.51	1.072	21.68	22.57	0.961

Table IV. Standard Deviations

	Cr	Ni
No. of samples	35	35
Maximum % concentration	32.21	24.53
Minimum % concentration	27.72	14.26
Spectrographic/chemical ratio		
Maximum	1.118	1.131
Minimum	0.947	0.907
Average	1.032	1.002
Standard deviation	0.034	0.042
% deviation	3.3	4.2

Table V. Precision and Reproducibility Test on a Single Sample

Per Cent Transmission						
Cr 3169	Ni 3087	Fe 3259	X	Y	% Cr	% Ni
14.6	19.6	49.2	1.835	0.624	28.35	17.68
15.5	22.1	51.3	1.770	0.631	28.80	18.17
12.4	17.2	44.5	1.796	0.605	28.80	17.42
13.9	19.2	48.0	1.790	0.616	28.75	17.70
15.4	22.1	52.7	1.760	0.612	29.05	17.78
16.4	22.6	53.6	1.790	0.635	28.59	18.16
15.0	21.0	51.3	1.793	0.616	28.75	17.70
14.0	19.8	50.0	1.778	0.602	28.99	17.45
17.8	24.4	57.7	1.813	0.621	28.52	17.72
17.4	23.6	56.0	1.835	0.629	28.38	17.83

σ Cr = 0.223 % deviation = 0.77
σ Ni = 0.237 % deviation = 1.33

Conclusions

The precision of the method is sufficient for the control of melting operations of stainless steel. The primary advantage claimed for the method is speed. Analyses have been made in this laboratory with total time of 9 minutes elapsed between taking the sample at the furnace and reporting the results.

A test of the reproducibility of results and the precision attainable was made by making a stepped-sector exposure, a series of exposures for standard samples, and ten exposures for a single sample on the same plate. The complete series of curves and constants were determined from this plate. The data for the ten determinations on the single sample are shown in Table V.

The per cent deviation for a series of seven determinations by chemical methods on the same sample used for the determinations listed in Table V was 1.71 per cent for chromium and 0.89 per cent for nickel. Obviously the spectrographic method compares favorably with the chemical method when the necessary care is taken in making a series of determinations.

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Presented before the Alabama Section of the American Chemical Society.

A Fused Salt Technique in Spectrochemical Analysis

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FREQUENTLY the chemical spectroscopist is called upon to analyze powdered substances for minor constituents. Broadly speaking, analyses of material in the solid state present more difficulties than determinations of constituents in solution. Substances which are soluble in water, acids, or other common solvents entail few problems; in such cases it is usually feasible to evaporate a drop of a solution of the material, containing an added internal standard when necessary, on the surfaces of smooth-faced graphite electrodes and to excite the residue in a high-voltage alternating current arc (*2*).

Substances which do not dissolve in the common solvents are generally treated according to the time-honored practice of vaporizing and exciting them from the cored anode of a direct current arc. As is well known, analyses carried out by means of the direct current arc often fall short of the accuracy which is possible in spectrochemical analysis. Not all of the blame can be laid to the intrinsic properties of the direct current arc, however. A common source of error, both in the preparation of standard powder samples and in the analysis of unknown samples, is lack of homogeneity. It is an exceedingly arduous task to disperse small amounts of added material uniformly throughout a matrix in the preparation of standard powders.

Confronted with this problem in the analysis of titanium dioxide, the authors have developed a method in which samples are fused with potassium bisulfate, and graphite electrodes are

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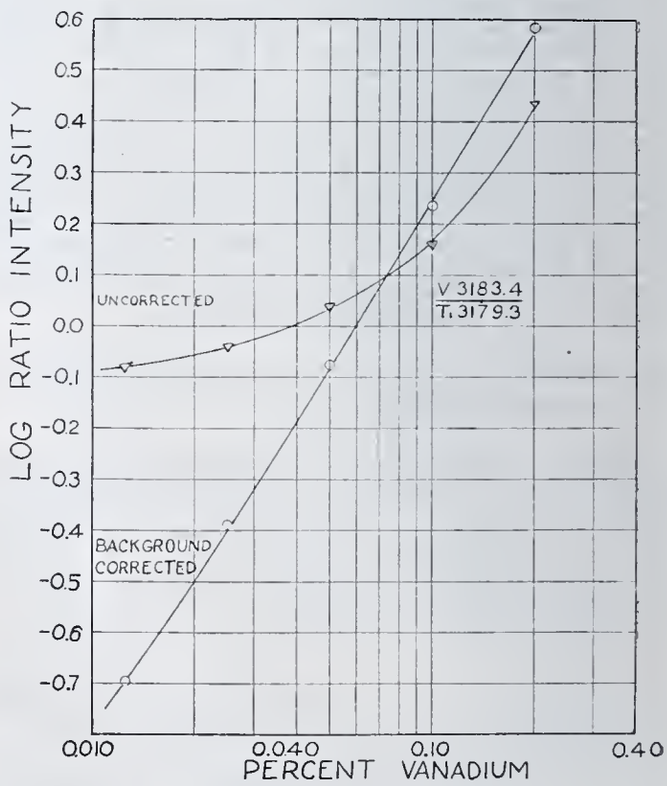

Figure 1. Working Curve for Vanadium in Titanium Dioxide

Table I. Typical Results of Titanium Dioxide Analysis

Antimony Added	Antimony Found	Chromium Added	Chromium Found	Vanadium Added	Vanadium Found	Iron Added	Iron Found
%	%	%	%	%	%	%	%
1.00	1.00	0.100	0.112	0.200	0.192	0.100	0.090
	0.67		0.073		0.171		0.114
	1.10		0.101		0.120		0.109
	0.95		0.111		0.150		0.111
	0.92		0.096		0.170		0.106
Av.	0.93		0.099		0.161		0.106
0.50	0.57	0.050	0.056	0.100	0.106	0.050	0.056
	0.48		0.045		0.097		0.046
	0.49		0.051		0.089		0.054
	0.55		0.045		0.091		0.050
	0.61		0.059		0.092		0.055
Av.	0.54		0.051		0.095		0.052
0.25	0.22	0.025	0.024	0.050	0.050	0.025	0.049
	0.28		0.025		0.043		0.022
	0.26		0.026		0.057		0.033
	0.29		0.025		0.040		0.019
	0.35		0.030		0.042		0.037
Av	0.28		0.026		0.046		0.032
0.12$_5$	0.15	0.013	0.014	0.025	0.025	0.0125	0.016
	0.15		0.012		0.024		0.013
	0.13		0.013		0.034		0.009
	0.05		0.008		0.022		0.012
	0.14		0.008		0.029		0.009
Av.	0.12		0.011		0.027		0.012
Av. error (5 detns.)	7.8		4.8		10.1		10.5

coated by dipping them in the molten solution. In addition to providing a simple means of preparing homogeneous standards, the procedure eliminates the undesirable sputtering characteristics of the titanium dioxide arc.

Preparation of Standard Samples

Exactly 100.0 grams of spectroscopically pure potassium bisulfate and 5.00 grams of pure titanium dioxide were fused together in a 115-mm. porcelain dish over a Méker burner. To the melt were added 0.0600 gram of antimony trioxide, 0.0352 gram of ferrous ammonium sulfate, 0.0240 gram of vanadous chloride, and 0.0141 gram of potassium dichromate. The resulting clear melt contained 1.00 per cent antimony, 0.20 per cent vanadium, 0.10 per cent iron, and 0.10 per cent chromium (all based on titanium dioxide). After the melt had been thoroughly stirred, smooth-faced graphite electrodes were dipped therein. The ends of the electrodes were barely touched to the surface of the melt. A smooth thin coat of salt was assured by preheating the electrodes before immersion of their tips, and by shaking off the excess salt immediately after their removal from the melt. A thin vitreous coating was found to lead to better reproducibility, since electrode separations could be more accurately controlled.

When the remaining melt had solidified, it was crushed and weighed. One half of the crushed salt was re-fused with 50.0 grams of potassium bisulfate and 2.50 grams of titanium dioxide. In similar fashion, succeeding standard melts and electrodes were prepared, each containing one half the impurity content of the former.

Spectra were recorded by a large quartz Bausch & Lomb Littrow spectrograph set for the region 2500 to 3400 Å. The electrodes, held in water-cooled nickel electrode holders, were accurately adjusted to a separation of 1.0 mm. and arced at 4.5 amperes (2200-volt alternating current arc). The exposure time was 1 minute (no pre-arc), with a spectrograph slit of 30 microns. Eastman Spectrum Analysis No. 1 plates, calibrated by means of an iron arc and a rotating stepped sector, were developed for 2 minutes at 18° C. in D-72.

Treatment of Data

The line pairs chosen for the construction of working curves were: Sb 2598/Ti 2590.3 Å., V 3183.4/Ti 3179.3 Å., Fe 3020.6/Ti 3002.7 Å., and Cr 3021.6/Ti 3002.7 Å. It was found desirable, in the interests of precision, to apply background corrections to all lines except Sb 2598 Å. and Ti 2590.3 Å., which lie in a region of low background. The background correction, discussed by Pierce and Nachtrieb (*3*), consists in converting line and background transmissions (or densities) into relative intensities, and subtracting the background intensities from the line plus background intensities for both analysis and internal standard lines. The logarithms of the background-corrected line intensity ratios (analysis line ÷ internal standard line) are then found to be a linear function of the logarithms of the concentrations down to rather low concentration limits. The fact that the working curves depart from linearity at low concentrations indicates that the titanium dioxide was not pure. The residual concentrations of impurities in the titanium dioxide could be determined by a method of successive approximations (*2*) or by construction of intensity-concentration graphs (*1*, *3*).

Figure 1 shows the effect of background correction on the vanadium working curve; the increased sensitivity (slope) occasioned by the correction is typical. Moreover, it is noteworthy

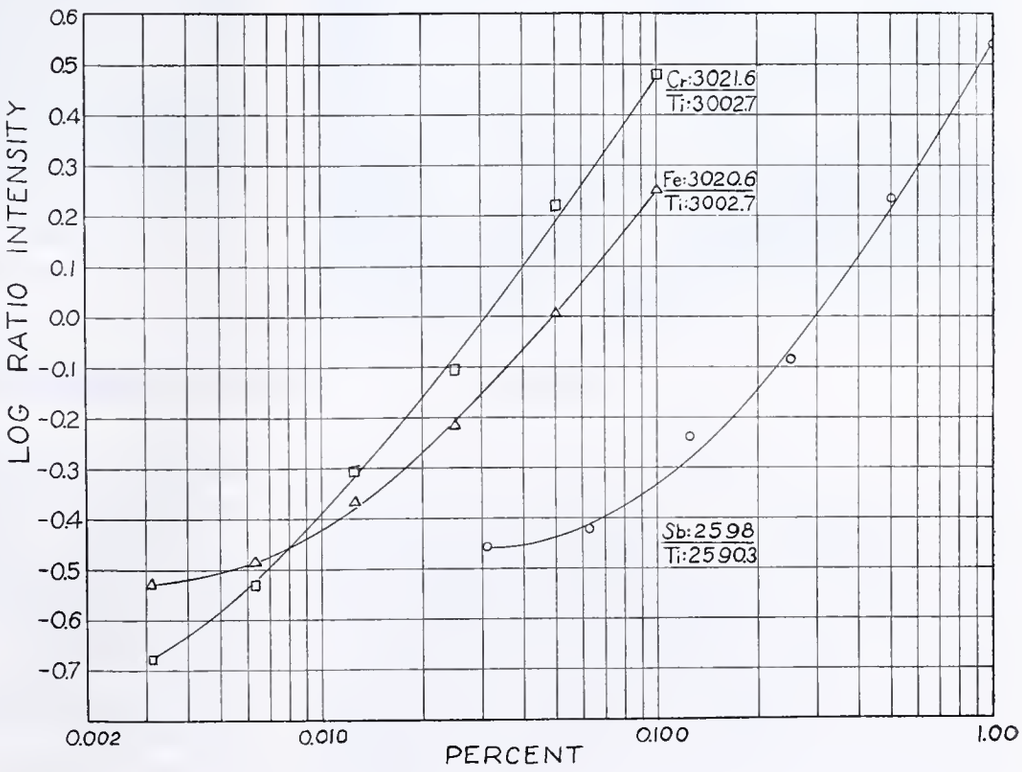

Figure 2. Working Curves for Iron, Chromium, and Antimony in Titanium Dioxide

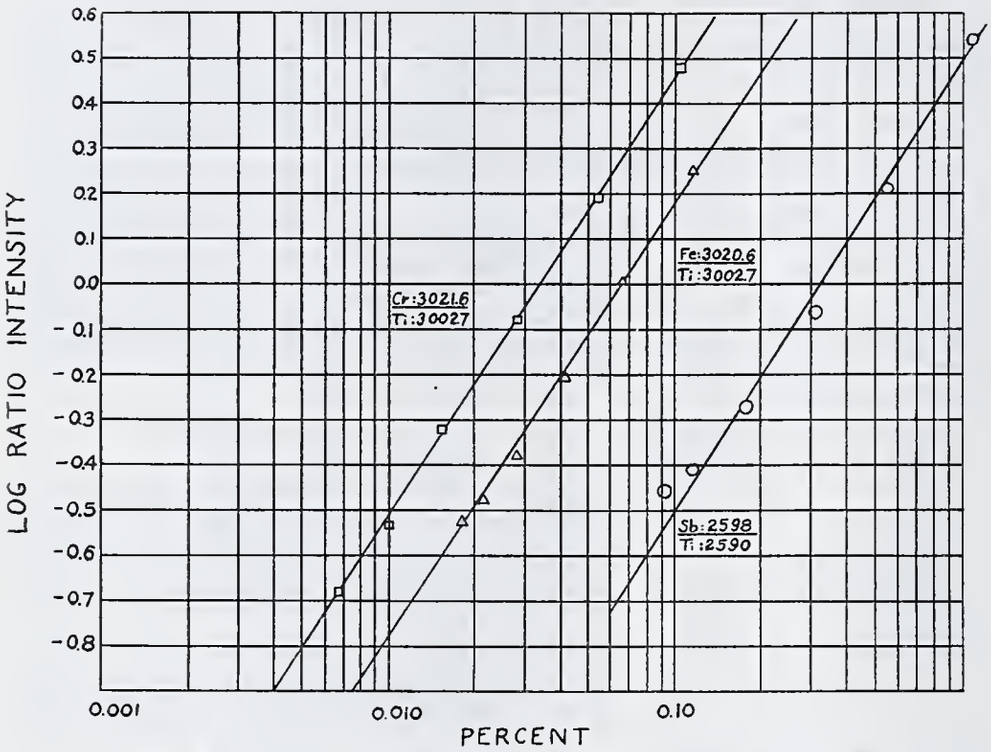

Figure 3. Working Curves Corrected for Residual Impurities

Table II. Comparison of Spectrochemical and Chemical Analyses of Iron in Titanium Dioxide

Sample No.	Iron Found Spectrochemical %	Iron Found Chemical[a] %
1	0.003	0.003
2	0.006	0.006
3	0.016	0.016
4	0.027	0.033
5	0.029	0.029
6	0.002	0.003
7	0.006	0.007
8	0.005	0.005
9	0.002	0.004
10	0.003	0.003
11	0.005	0.004
12	0.004	0.004
13	0.009	0.011
14	0.004	0.006
15	0.009	0.012
16	0.006	0.006

[a] Colorimetric thiocyanate method.

that the slope of the background-corrected vanadium working curve is 1.063, in good agreement with the theoretically predicted value of unity. Figure 2 shows the working curves obtained for antimony, iron, and chromium. Figure 3 shows the final working curves, obtained from the curves of Figure 2 by correction for the residual impurities in the titanium dioxide.

Table I lists the results of four analyses in quintuplicate for each of the elements, together with the average deviation from the true values. The time required for the eighty determinations was about 5 hours.

Table II gives a comparison of results for iron in sixteen samples of titanium dioxide by chemical analysis and by the spectrochemical procedure described in this paper.

Summary

A technique has been described for the analysis of powders which may be dissolved in molten potassium acid sulfate. The method has been applied to the determination of antimony, vanadium, chromium, and iron in titanium dioxide, analysis conditions and working curves for which are presented. The advantages derived from the application of background corrections are illustrated by working curves for a vanadium line.

The fused salt technique of preparing samples and standards may be extended to many other substances which are difficult to dissolve in the common solvents or to arc as such. The selection of a flux in a particular case will be determined largely by solubility considerations and by its suitability as a spectroscopic buffer.

Acknowledgment

The authors wish to express their appreciation to Leavitt Gard and to John Darby for carrying out the chemical analyses required in the testing of the method.

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Identification of Rust on Iron and Steel

Sir: In a recent article [Ind. Eng. Chem., Anal. Ed., 15, 464 (1943)] I described a sensitive test for iron rust which involved the use of gelatin-coated paper. Processed, single-weight, glossy photographic paper was recommended for the test. It has been called to my attention that two nonsensitized gelatin papers, Eastman Kodak Imbibition Paper and Defender Backing S, are available and my reasons for not using such papers were questioned.

The two nonsensitized papers mentioned are available in double-weight stock only. I tried double-weight papers in the original tests and found that they did not adhere well to the surface being tested unless constant pressure was applied. The single-weight paper described in the article adhered perfectly without pressure, even when applied to curved surfaces of short radius.

I still prefer the desensitized single-weight paper for the reason given. In all other respects the double-weight nonsensitized papers are satisfactory. I regret that I neglected to mention this point in the article.

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Determination of Permeability of Agricultural Spray Coatings to Water Vapor

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A simple empirical method capable of yielding reproducible permeability measurements on films formed from wax and oil-spray emulsions of the oil-in-water type is described. The experimental conditions are easily duplicated. For the type of film studied a linear relationship exists between moisture impedance and film thickness. Advantages in the use of paraffin wax are demonstrated and the role of bentonite in the emulsion is indicated.

IN THE course of studies on the development and use of emulsions for spraying plant materials to retard desiccation, it became necessary to determine the efficiency of films formed from such spray materials in reducing the passage of water vapor. Information on the interrelationships of film permeability, film thickness, and the chemical composition of the emulsion is indispensable for the formulation of spray materials having desirable physical characteristics. Methods described in the literature were not directly applicable to the type of material under consideration here from the point of view of preparation of the test film, precision of the measurements, and reproducibility of experimental conditions. It was therefore necessary to develop a more satisfactory procedure.

Examination of the literature (*3*), as well as laboratory experience, has indicated the many difficulties encountered in obtaining reproducible values from what appears to be a simple physical measurement. Uncertainties may be due to irregularities in the test film or to lack of consideration of the factors involved in the permeability measurement. In the method herein presented these difficulties have for the most part been eliminated by an exacting uniformity in procedure. The method is a simple one, and although designed for a specific kind of surface covering, it should be generally applicable to measurements on various types of coatings, perhaps with slight modification of detail in some cases. The apparatus required is readily available to most laboratories and if all precautions in technique are observed there should be no difficulty in obtaining reliable comparative measurements.

The passage of water vapor through a film has been considered as an ordinary diffusion process, in which case the following relation obtains:

$$M = kAt\frac{(P_1 - P_2)}{l} \qquad (1)$$

where M = mass of water vapor that passes through the film
A = area of film surface
t = time
$P_1 - P_2$ = water vapor pressure differential across the film
l = film thickness
k = constant for the film material

In actual practice, however, Equation 1 is too simple to express the relationship between the variables. This matter has been recognized and discussed by many workers (*1, 2, 4, 8, 9*). Since the interest here was primarily in obtaining reliable comparisons between the permeabilities of various samples, no attempt was made to establish the validity of Equation 1 with respect to all the variables; rather, emphasis has been placed upon a study of the precision of permeability measurements as a function of experimental technique with a view towards arbitrary standardization of conditions. It was desirable that the experimental conditions be easily maintained and readily capable of duplication, and that factors which might cause uncertainties in the results be controlled. Such considerations led to a satisfactory procedure.

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Experimental

PERMEABILITY MEASUREMENT. A critical survey of procedures used for this type of measurement up to 1937 has been published by Carson (*3*) and the more recent experimental methods have been reviewed by Lishmund and Siddle (*9*). In the method reported here the material to be tested is fastened over the mouth of a cup containing water and the loss of moisture through the sample to a drier atmosphere is determined by weighing.

The Payne permeability cup (*10*), which is available commercially, was used for the measurements. It was found convenient, however, to replace the usual clamps with steel ones, since under constant usage the threads on the originals were easily stripped. Desiccators of the type shown in Figure 1, with an inside diameter of 250 mm., were used. The cups are supported within the desiccator by a 1.9-cm. (0.75-inch) plywood board which accommodates four cups and has a receptacle for desiccant in the center (see Figure 1). The cup support has an outside diameter of 233 mm.; the diameter of the center receptacle, which is made of metal, is 100 mm., and it is 7 to 8 mm. deep. The holes for the cups are 40 mm. in diameter and are symmetrically placed, the center of each hole being 25 mm. from the outside edge. The relation between cup position and the surface of the desiccant is then essentially the same for each determination.

FIGURE 1. PERMEABILITY APPARATUS
Cup support containing desiccant, permeability cup, and loaded desiccator

Dowflake (a technical grade of calcium chloride obtainable from the Dow Chemical Company) was found satisfactory as the desiccating agent. It is necessary to use fresh desiccant in the center of the cup support for each run and it is recommended that the Dowflake for this purpose be stored in small completely filled and tightly closed bottles, each containing enough desiccant for the center compartment (about 57 grams). The Dowflake in the lower part of the desiccator is changed about once a week; this desiccant is contained in a metal receptacle for convenience and cannot be seen in the illustration. The desiccators themselves are kept in an air thermostat, at 25 ± 0.4° C. The thermostat is near the balance and is constructed so that the

desiccators need not be removed from it for the placing or removal of the cups.

Preliminary trials indicated that reproducible results could be obtained only if experimental conditions were constant. For this reason the following schedule was strictly maintained. Unless otherwise noted all measurements were made on the basis of a time period of 150 minutes; for the type of material studied no significant error was introduced by lack of attainment of the steady state of moisture interchange.

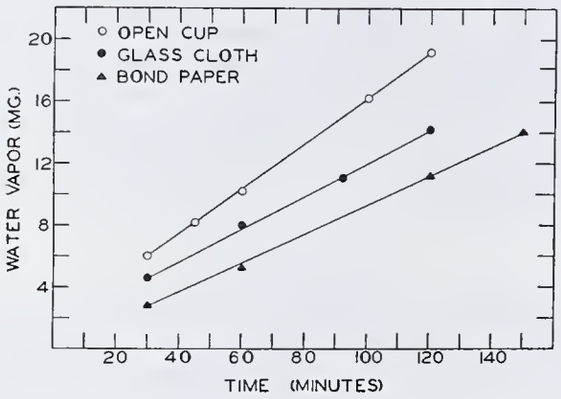

Figure 2. Permeability of Glass Cloth and Bond Paper

Before the determination is started, distilled water, the small bottles of desiccant, and the cups are brought to 25° C. For each run, four samples of the film are used, three for the actual measurements and one for the counterpoise. A 5-ml. sample of distilled water is pipetted into each of three cups. Since all cups are of equal dimensions, the distance between the water surface and the film support is always the same. A film disk, with the coated side towards the dry atmosphere, is placed between the flanges of each of the four cups and the flanges are then clamped together tightly. The cup containing no water is used as the counterpoise. Fresh Dowflake is then placed in the center receptacle of the cup support inside the desiccator.

Starting at 0 minutes the first cup is weighed, at 1 minute the second and at 2 minutes the third; at 4 minutes the four cups are placed in the desiccator and the cover is replaced. At 154 minutes the cups are removed from the desiccator; at 156 minutes the first cup is weighed again and the others at 157 and 158 minutes, respectively. The cups, then, have been exposed to the dry atmosphere for 150 minutes and to the air for 6 minutes; the only difference in cup treatment is that the first is in the air 4 minutes before being in the desiccator and 2 minutes afterward, the second cup 3 before and 3 afterward, and the third cup 2 before and 4 afterward. With very impermeable materials it may be necessary to use a longer time period; in this case the exact timing becomes of less importance. The desiccator should not be opened during the run.

In all cases determinations were made in triplicate and the values usually agreed within ±3 per cent. It was necessary to use a counterpoise identical with the loaded cups except for the water, to eliminate any extraneous changes in weight. Experiment showed that the leakage between the sample and the rim of the cell or through the cut edges of the film support was so small as to be within the limits of experimental error for the type of material studied and no correction was applied. When measurements are made on relatively impermeable materials it may be necessary to use gaskets or other means of reducing rim and edge leakage.

Preparation of Films. Satisfactory free films could not be prepared and a supporting material had to be employed. Glass cloth (*10*) and various types of paper were tried; the most consistent results were obtained with bond paper (Howard Bond, white, Sub. 24) and this was used for the work reported here. The water-marked parts of the sheet were not used.

The doctor blade technique could not be used because of the buckling of the film support caused by water absorption from the emulsion, and spraying or dipping yielded irregular films. Satisfactory films were prepared by the spinning method suggested by Gardner and Sward (*7*) and Gardner (*6*). The apparatus consisted essentially of a 7.5-cm. circular spinning table powered by a 0.125-horsepower motor. The speed could be varied by means of pulleys in steps from 430 to 3480 r. p. m. The film support was secured to the spinning table, covered with the solution from which the film was to be formed, and then spun for 30 seconds. Experiment indicated that the film thickness did not vary with the time of spinning between 10 and 500 seconds. The film was allowed to dry in air before measurements were made or before a second coat was applied.

The thickness of the film support was measured with a micrometer before spinning, and after the film had dried another measurement was made; the film thickness was then obtained by difference with an estimated accuracy of ±3 microns. The thickness value for each film was the average of three measurements halfway between the center and the outer edge of the test disk. All reported results represent the average of values obtained from 3 disks. In the case of viscous solutions the films were thicker at the center, but this was usually not significant. The film weight was determined by difference from the weight of the supporting disk and the weight of the disk plus the dried film; the values represent the weight of film covering the total area of the test disk (24 sq. cm.).

Table I. Variation of Moisture Impedance, Film Thickness, and Film Weight with Speed of Spinning

(Oil emulsion)

Speed	Moisture Impedance	Film Thickness	Film Weight
R. p. m.	*Hr. sq. cm./mg.*	*Microns*	*Mg.*
2270	0.43	49	119
1760	0.49	62	158
1390	0.55	82	207
1070	0.59	94	228
880	0.70	120	295

Experimental Results

The Payne permeability cup is designed to present an area of 10 sq. cm. for the passage of the water vapor. Although it has not been shown that the permeability is linearly proportional to the area, the results in this paper have been calculated on the basis of 1 sq. cm. for convenience in expression; since all data were obtained under the same conditions, no error in comparative results should be introduced by this procedure. Likewise the results have been calculated on the basis of water loss per hour, although unless otherwise noted the actual time period for the measurement was 150 minutes. The permeability is expressed therefore as milligrams of water vapor permeating through 1 sq. cm. area of film and supporting material in 1 hour under the specified experimental conditions. Where the results have been expressed as the reciprocal of the permeability, or the moisture impedance, the units are hour square centimeters per milligram. The reciprocal relation between moisture impedance and moisture permeability is analogous to that between electrical resistance and electrical conductivity. In any system, then, the impedance values should be additive while the permeabilities

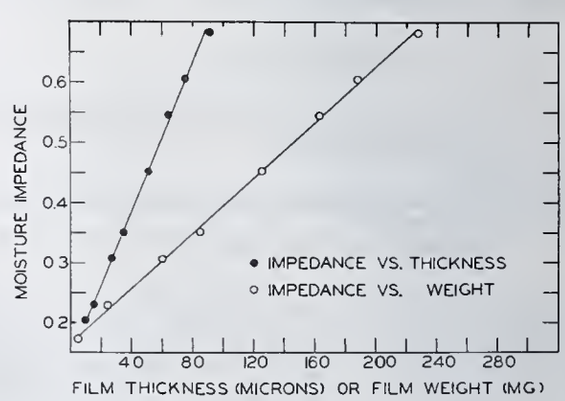

Figure 3. Moisture Impedance *vs.* Film Thickness and Film Weight

Oil emulsion

Table II. Oil Content and Moisture Impedance

(Oil emulsions)

Oil %	Moisture Impedance *Hr. sq. cm./mg.*
9	0.23
16	0.23
31	0.75
47	3.47

Table III. Effect of Wax on Moisture Impedance, Film Thickness, and Film Weight

(Oil emulsions)

Oil and Wax	Moisture Impedence *Hr. sq. cm./mg.*	Film Thickness *Microns*	Film Weight *Mg.*	Moisture Impedance per Unit Film Thickness
21.6% oil No wax	0.26	10	26	0.026
20.9% oil 0.7% wax	1.04	16	36	0.065
20.2% oil 1.4% wax	1.13	20	44	0.057
16.5% oil 5.1% wax	1.25	20	38	0.063

are not, just as in an electrical circuit the resistances are additive but the conductances are not. This has been discussed by Edwards (*5*).

Figure 2 presents the rate of loss of water vapor from the open cup as compared with that through glass cloth and bond paper. The ratios of the open cup values to those of the glass cloth agree closely with the numerical values of Payne (*10*). The permeability value obtained by extrapolation of the curves to zero time is probably due to the water loss during the 6-minute exposure to the air. The fact that the curve is linear up to a water vapor loss of almost 20 mg. from the open cup indicates that the efficiency of the desiccant has not been decreased by the absorption of that amount of moisture.

Table I presents the moisture impedance, thickness, and weight of a film formed from an oil-emulsion spray as a function of the speed of spinning. If these data are plotted it is apparent that the film thickness and weight are essentially linear with the moisture impedance.

Figure 3 presents the moisture impedance curves of films formed from an oil-emulsion spray. In this case the variation was obtained by changing the dilution of the emulsion rather than the speed of spinning. The linearity is in agreement with the results of other workers for some types of films under certain conditions (*11*, *13*). Wing (*12*) has reported the impedance of paint or enamel films to liquid water to be a linear function of film thickness. The moisture impedance obtained by extrapolation to zero thickness or weight agrees closely with the value for the film support alone. This type of information is important, since after linearity between moisture impedance and film thickness has been established for a given emulsion it is possible to interpolate the impedance at any desired thickness for comparative purposes.

Figure 4 presents the moisture impedance–per cent solids curves for the films formed from three different emulsions. Here also the impedance values approach that of the support material as the lower limit. The relative efficiency of these emulsions in retarding the passage of water vapor is obvious. At 12 per cent solids, wax emulsion A is about twice as efficient as emulsion B and 4 times as efficient as the oil emulsion; at 20 per cent solids, the efficiency ratios are 1.7 and 8.5, respectively.

Table II presents the moisture impedance of films formed from oil-emulsion sprays as a function of the oil content. It was necessary to increase the oil content to about 30 per cent to obtain a threefold increase in the impedance over the values obtained when less than 16 per cent oil was used. Increasing the oil content from 31 to 47 per cent gave over a fourfold increase in the impedance, but this range is not of practical interest because sprays containing such high percentages of oil tend to cause injury to the plants.

Table III shows the effect of incorporating small amounts of paraffin wax in an oil emulsion; it required only 0.7 per cent wax to increase the impedance fourfold, a more economical means of obtaining a greater impedance than the use of higher percentages of oil. There was an increase in the impedance per unit film thickness due to the presence of the wax.

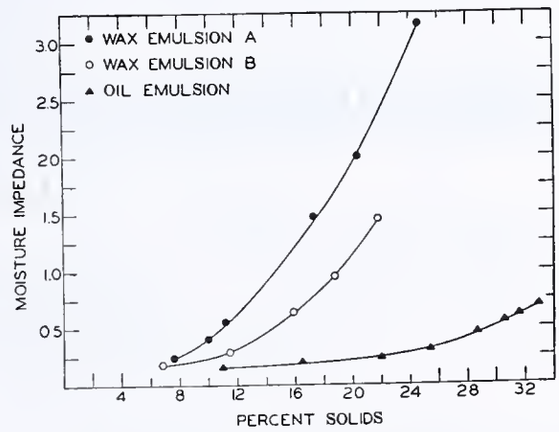

Figure 4. Moisture Impedance *vs.* Per Cent Solids

Table IV shows the effect of adding bentonite to an oil-emulsion spray. The bentonite increases the viscosity of the emulsion, and since the films are prepared under standard conditions, it is evident that an increased film thickness results. At the higher bentonite levels, however, the impedance per unit film thickness was markedly decreased. At the lower bentonite levels the decreased impedance per unit thickness was counterbalanced by the increased thickness, so that there was no net change in the impedance values in going from 0 to 2.1 per cent bentonite. Above 2.1 per cent bentonite the film thickness became the predominating factor and the over-all impedance values were increased.

The formulation of emulsions of given physical properties has been facilitated by information obtained from the above type of experiment.

Table IV. Effect of Bentonite on Moisture Impedance and Film Thickness

(Oil emulsions)

Bentonite %	Moisture Impedance *Hr. sq. cm./mg.*	Film Thickness *Microns*	Moisture Impedance per Unit Film Thickness
0.0	0.21	11	0.019
0.5	0.21	14	0.015
2.1	0.22	19	0.012
4.2	0.28	63	0.0045
5.2	0.47	125	0.0038

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Determination of Nicotine and Nornicotine in Tobaccos

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To determine nicotine and nornicotine in tobacco the total steam-volatile alkaloids are isolated by steam distillation from a strongly alkaline solution containing sodium chloride in excess. One aliquant of the distillate is treated with silicotungstic acid precipitating nicotine and nornicotine, and another is treated with nitrous acid, steam-distilled from an alkaline solution, and the nicotine precipitated as a silicotungstate. The nornicotine is determined by difference. Analysis of tobaccos differing widely in alkaloid content shows the rather common occurrence of nornicotine. Comparison with nicotine determinations by the conventional methods shows the unreliability of the heretofore accepted method of analysis when nornicotine is present.

A METHOD of analysis for nicotine and nornicotine when present together in solution has been presented by Markwood (*5*). It was developed for cases in which the alkaloids are already separated from interfering materials, and no procedure is given for obtaining the alkaloids from tobacco or tobacco products. To separate nicotine and nornicotine from the plant material, a steam distillation or continuous extraction with an organic solvent such as benzene is necessary. In view of the difficulty experienced in completely removing nornicotine from alkaline plant material by repeated extraction with benzene, ether, or gasoline, the method presented here resorts to a steam distillation, which completely separates both the nicotine and nornicotine from the sample.

This method is based on the assumption that anabasine or other steam-volatile secondary-amine alkaloids aside from nornicotine are absent; such alkaloids have been found only in traces in *Nicotiana tabacum*. The steam-volatile secondary-amine alkaloid has been identified as nornicotine by methylation in most of the samples analyzed.

The nornicotine is determined as the difference between the total steam-volatile alkaloid and the nicotine; consequently the analysis takes place in two parts. The first is an application to nornicotine of Burkat's method (*4*) for determining anabasine; it depends upon the complete steam distillation of nicotine and nornicotine from strong alkali solutions saturated with sodium chloride. The second part, which has been proved satisfactory by Markwood (*5*), involves treating the solution containing both alkaloids with nitrous acid and steam-distilling from an alkaline solution, whereby the secondary amines, including nornicotine, remain behind as nitrosoamines and the nicotine is obtained in the distillate.

Analysis of Standard Solutions

The standard solution of nicotine used for proving the method was prepared from a sample of nicotine that had been vacuum-distilled, treated with nitrous acid to remove any secondary amine, and then steam-distilled from an alkaline aqueous solution.

The nornicotine used in preparing the standard nornicotine solution was identical with that used by Markwood (*5*), had been obtained from Robinson Medium Broadleaf, a strain of Maryland tobacco, and formed a picrate melting at 190–191° C. The absence of nicotine was established by treating a sample with nitrous acid and steam-distilling the aqueous solution after making it basic to phenolphthalein. No alkaloid was present in

Table I. Recovery of Nicotine and Nornicotine in Mixtures by Steam Distillation from Strong Alkali and Sodium Chloride

Composition of Mixture		Silicotungstate Residue[a]		
Nicotine	Nornicotine	Direct pptn.	Pptn. of distillate	Recovery
Mg.	*Mg.*	*Mg.*	*Mg.*	%
18.2	2.6	190.1	184.8[b]	97.2
14.5	4.5	172.7	170.3	98.6
10.9	6.2	152.2	155.0	101.8
7.7	7.2	138.9[b]	137.1	98.7
4.5	8.2	118.6[b]	117.9	99.4
				Av. 99.1

[a] Average of three determinations except where otherwise noted.
[b] Average of two determinations.

Table II. Analysis of Known Solutions of Nicotine-Nornicotine Mixtures

Nicotine			Nornicotine		
Calcd.	Found	Recovered	Calcd.	Found	Recovered
Mg.	*Mg.*	%	*Mg.*	*Mg.*	%
23.50	23.47	99.9	...	...	...
16.80	16.52	98.3	2.58	2.62	101.6
14.93	14.43	96.7	3.65	3.67	100.6
7.83	7.71	98.5	7.32	7.23	98.8
...	...	...	10.90	10.40	95.4
	Av.	98.4			99.1

the distillate. This nornicotine was steam-distilled and the distillate used in preparing the standard solution. Both solutions were standardized by the silicotungstic acid method.

The reliability of the silicotungstate precipitation for the determination of nornicotine is evidenced by the data presented in Table I.

The conventional method (*1*) for the steam distillation of nicotine specifies the use of a slight excess of alkali. When nornicotine is present, the time for complete distillation of the alkaloids is long and the volume of distillate consequently large, because the nornicotine distills only slowly from a weakly alkaline solution. When the distillation is made from a strongly alkaline solution, the speed of removal of alkaloids is greatly increased, and the addition of sodium chloride further hastens the process. Avens and Pearce (*2*) report that in their nicotine procedure excessively large amounts of sodium hydroxide give abnormally high results, but that they are due to something besides nicotine. This material is undoubtedly nornicotine, which did not distill readily at the lower alkali concentration. A comparison of the weights of the alkaloid-silicotungstate ignition residues of nicotine-nornicotine mixtures obtained by direct precipitation and by precipitating after steam distillation from strong alkali and sodium chloride (Table I) shows that an average recovery of 99.1 per cent may be expected. The alkali should be 30 per cent by weight and the sodium chloride in excess in order to have a saturated solution at all times during the distillation. When the fresh distillate and the residue from some of the samples were tested, the absence of alkaloid was indicated by no precipitation with silicotungstic acid.

When mixtures of the standard nicotine and nornicotine solutions were analyzed, one aliquot was steam-distilled directly to get all the steam-volatile alkaloid in the distillate, while another aliquot was treated with nitrous acid and then alkali before steam distillation. The alkaloids in the distillates were precipitated as silicotungstates and the precipitates ignited. The nornicotine was calculated from the difference in weights of the residues. The results shown in Table II indicate that such a procedure is satisfactory.

Analysis of Tobacco for Nicotine and Nornicotine

A 2.5-gram sample of powdered tobacco, a small piece of paraffin, 10 ml. of sodium hydroxide (30 per cent by weight), and 10 grams of sodium chloride are placed in the inner chamber of an improved steam-distillation apparatus (*3*) and steam-distilled into 3 ml. of dilute hydrochloric acid (1 + 4). Distillation is continued until a few drops of fresh distillate fail to give an opalescence when tested with silicotungstic acid solution. The distillate is concentrated by boiling (boiling stones added to prevent bumping) until the volume is less than 25 ml., and then transferred to a 25-ml. volumetric flask. Washings are added to the flask, and the liquid is brought to the mark. A 10-ml. aliquant is taken for precipitation of the nicotine and nornicotine with silicotungstic acid solution (12 per cent) according to the A. O. A. C. procedure for nicotine (*1*). The silicotungstate precipitate is filtered through a tared Gooch-type crucible and ignited, giving residue A. Another 10-ml. aliquant is made neutral to phenolphthalein, and 2 ml. of acetic acid (30 per cent) and 0.5 gram of solid sodium nitrite are added (*5*). After standing at room temperature for 20 minutes, this solution is made slightly alkaline with sodium hydroxide and steam-distilled into 3 ml. of hydrochloric acid from the improved distillation apparatus as used above. The distillate contains the nicotine, which is precipitated, filtered, and ignited as for the first aliquant, giving residue B.

The nicotine is calculated from residue B and the nornicotine from the difference between residues A and B. The factor 0.1042 has been assigned to the nornicotine calculation. This factor was obtained as the theoretical value and was substantiated by use in the analysis of standard solutions of nornicotine.

Weight of residue B × 0.1140 = weight of nicotine present in aliquant

(Weight of residue A − weight of residue B) × 0.1042 = weight of nornicotine in aliquant

Table III. Steam-Volatile Alkaloids in Analyzed Samples of Tobacco and Commercial Nicotine Sulfate

Sample	Calculated as Nicotine and Nornicotine, Proposed Method			Calculated as Nicotine	
	Nicotine	Nornicotine	Total	Proposed method	A. O. A. C. method[a]
	%	%	%	%	%
Nicotiana tabacum					
Robinson's Maryland Medium Broadleaf, Sample 1[b]	0.34	1.71	2.05	2.16	0.76
Cash, flue-cured[c]	0.70	2.40	3.10	...	...
Robinson's Maryland Medium Broadleaf, Sample 2[c]	0.98	2.16	3.14	3.32	1.77
Burley, Halley[c]	1.23	1.41	2.64	2.78	2.14
Turkish, Xanthi, American grown[c]	6.59	1.28	7.87	...	...
Maryland-Connecticut Broadleaf[c]	2.22	0.49	2.71	2.75	2.78
N. rustica					
Sample 1	4.57	0.99	5.56	...	...
Sample 2 (Brasilia)	6.71	0.66	7.37	...	...
Commercial nicotine sulfate					
Sample 1[c]	34.75	4.54	39.29	39.70	39.70
Sample 2	38.82	1.10	39.92	40.02	39.75

[a] Determined elsewhere.
[b] Source of nornicotine used to prepare standard solution.
[c] Steam-volatile secondary-amine alkaloid identified as nornicotine by methylation.

The results of analyses of several samples of tobacco (*Nicotiana tabacum*), *N. rustica*, and commercial nicotine sulfate by this method are given in Table III. For some of the samples the table gives the total steam-volatile alkaloid calculated as nicotine both by the proposed method and by the A. O. A. C. method. Upon calculation of the alkaloids as nicotine and nornicotine, an unexpectedly widespread occurrence of nornicotine is indicated. It is also evident that when nornicotine is present the accepted method of analysis cannot be depended upon to give reliable results.

Although the improved steam-distillation apparatus was used in the proposed procedure, the conventional steam-distillation apparatus may be substituted with a resulting increase in time required for distillation.

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Sensitive Indicator for Volumetric Determination of Boiler Feedwater Alkalinity

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IN A lime-soda process for the treatment of raw water for boiler consumption, accurate laboratory tests on the effluent water provide the fundamental bases for efficient softener control. Volumetric determinations of treated water alkalinities form a most important phase of such laboratory tests, and the chemist should have available accurate and positive color indicators in order to make the analyses with the highest degree of precision.

An ideal indicator would show a sharp point of color transition coincident with the equilibrium point for the completion of the neutralization reaction. This point was computed for boiler feedwaters at about 4.89 by interpolating the data reported by Cooper (*2*) to a millimolarity of 0.333, the average sodium carbonate content of such waters.

The use of methyl orange for alkalinity titrations of water has received almost universal application and has been adopted as standard (*1*). This indicator, however, possesses a color change rather difficult to detect and, more important, has been shown by Cooper (*2*) to have a pH color response in disagreement with the equivalence point for complete neutralization.

The reduction of such indicator uncertainty has been reported by various investigators through the application of mixtures of indicators and of indicators and dyes, and these mixtures are described in the literature (*5*). In the latter type of mixture, the dye is added to supplement the color of the indicator and produce a color other than that of the indicator itself. For example, an inert dye, xylene cyanole FF, has been proposed by Hickman and Linstead (*3*) to augment methyl orange, giving a green color with the alkaline yellow of the indicator and a magenta red color with the acid orange. The application of this mixture to feedwater titrations is subject to criticism in that the pH of the color transition is 3.8, according to Willard and Furman (*7*), making the indicator less suitable than methyl orange itself.

Johnson and Green (*4*) have investigated the use of an alcoholic solution of methyl red and alphazurine (*6*) for alkalinity determinations, and this mixture was applied as an indicator in titrating treated water alkalinities at the laboratory of the Federal Central Heating Plant, Washington, D. C. A modification in the ratio of dye to indicator was made to improve the color transitions. The mixture showed great promise, but after standing several weeks the methyl red separated from solution and the color response of the indicator became less pronounced. These difficulties were eliminated by substituting the sodium salt of methyl red (dimethylaminobenzene sodium carbonate) in place of the methyl red and water in place of the alcohol. The aqueous solution was found to be stable and its use avoided the necessity of using alcohol. The indicator was prepared by dissolving 0.45 gram of methyl red sodium salt and 0.55 gram of alphazurine in 1 liter of distilled water. The pH value of the solution was observed to be approximately 7.3.

The indicator was checked for accuracy by titrating 100 ml. of a solution of sodium carbonate (*M* 0.00033) with 0.033 *N* hydrochloric acid, using the usual "double end-point" method with 3 drops of phenolphthalein indicator for the first end point and 3 drops of the prepared indicator for the second end point. Potential changes were measured with a commercial glass electrode vacuum-tube pH meter (Table I). Color transition and maximum potential deflection were observed to be in good agreement with the computed equivalence point for the neutralization reaction, and the volume of acid required for the second titration end point was double that required for the first end point, all of which indicated accurate results.

The pH values of the indicator color changes were determined in a series of adjusted solutions of potassium acid phthalate (0.02 *M*) and showed slightly lower values—viz., 4.8 for the gray and 4.6 for the purple gray. However, in dilute unbuffered feedwaters, this small discrepancy should not materially affect the accuracy of the determinations.

The sensitivity of the indicator is evidenced by a color change from green gray to gray with the addition of one drop of acid (approximately 0.05 ml.), and gray to purple gray with a second drop. The color transitions are sharp and distinct and show sufficient contrast to make the changes easily distinguishable. The gray intermediate color gives ample warning of the approaching purple gray which is used as the end point of the titration.

The effect of neutral salts on the indicator was determined by titrating with 0.03 *N* hydrochloric acid several prepared feedwaters containing large quantities of such salts. The solutions used in the experiment were (1) 100 ml. of 0.00033 *M* sodium carbonate containing no salts, (2) 100 ml. of 0.00033 *M* sodium carbonate containing 400 p. p. m. of sodium chloride, (3) 100 ml. of 0.00033 sodium carbonate containing 400 p. p. m. of sodium sulfate, and (4) 100 ml. of 0.00033 *M* sodium carbonate containing 400 p. p. m. of sodium chloride and 400 p. p. m. of sodium sulfate. The titration of all four solutions to the indicator end point (purple gray) required 2.00 ml. (±0.025 ml.) of 0.033 *N* hydrochloric acid. The pH values for these end points ranged from 4.6+ for solution No. 1 to 4.9+ for solution No. 4. These results showed that although neutral salts increased the apparent pH (as indicated by the glass electrode) of the equivalence point by as much as 0.3 pH unit, the stoichiometric end point, as indicated by the color response, was not affected, since all four neutralizations required the same volumes of acid within experimental error. Hence the indicator appears suitable for titrating any feedwater containing salts within the limits shown

TABLE I. NEUTRALIZATION OF SYNTHETIC FEEDWATER

(100 ml. of 0.00033 *M* Na_2CO_3 titrated with 0.033 *N* HCl at 20° C.)

	0.033 *N* HCl Added *Ml.*	pH	ΔpH	Indicator	Observed Color
	0.0	9.87	...	Phenol-phthalein	Red
	0.1	9.78	0.09		
	0.2	9.70	0.08		
	0.3	9.60	0.10		
	0.4	9.50	0.10		
	0.5	9.37	0.13		
	0.6	9.23	0.14		
	0.7	9.04	0.19		
	0.8	8.77	0.27		
	0.9	8.40	0.37		Pink
E. P.	1.0	7.50	0.90		Colorless
	1.1	7.15	0.35	Alphazurine-methyl red sodium salt	Green
	1.2	6.85	0.30		
	1.3	6.50	0.35		
	1.4	6.20	0.30		
	1.5	6.00	0.20		
	1.6	5.76	0.24		
	1.7	5.59	0.17		
	1.8	5.40	0.19		
	1.9	5.18	0.22		Green gray
E. P.[a]	2.0	4.68	0.50		Purple gray
	2.1	4.20	0.48		Purple
	2.2	3.75	0.45		
	2.3	3.56	0.19		

[a] 2 ml. of 0.033 *N* HCl is the stoichiometric as well as the actual volume (±0.05 ml.) required for neutralization.

above, and most natural waters in this country are normally within these limits.

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Determining the Mechanical Stability of Emulsions

A Rapid Quantitative Method

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A rapid quantitative method for determining the mechanical stability of emulsions involves measuring the rate of separation of internal phase under a constant centrifugal force. The reciprocal of the initial rate of separation at a constant centrifuge speed has been taken as a quantitative index of the mechanical stability of the emulsion. The method has been applied to both water-in-oil and oil-in-water emulsions stabilized by lecithin, soaps, and vegetable gums. It gives results in a few hours apparently comparable to those obtained by more tedious methods involving other factors and requiring measurements over many months.

Definite effects of the age of the emulsion on its mechanical stability as determined by this method have been found in the case of soap and saponin-stabilized emulsions.

In spite of the great importance of emulsions in many industries at the present time and the recent rapid increase in the number of new emulsifiers commercially available, very few quantitative data are given in the literature comparing the stability of various types of emulsions stabilized by different agents. This scarcity of numerical data is probably due to the lack of rapid quantitative methods for measuring the stability of emulsions.

The concept of emulsion stability involves the resistance of the emulsion to destruction by various forces. Probably the best measure of stability is the rate of separation of internal phase (*9*). The forces involved in any practical case depend on the conditions to which the emulsion is to be subjected during use.

This paper reports the development of a method for determining the resistance of emulsions to breaking under the mechanical stress of centrifugal force. Other destructive forces which might be used are shaking, stirring, jarring, impact, heating, and freezing and subsequent thawing. Probably the resistances of a series of emulsions will vary in the same manner for different types of mechanical stresses. However, for practical purposes it is advisable always to determine the stability of the emulsion under conditions of use in order to ascertain whether a more rapid method than conditions of use is a valid measure.

Numerous methods have been used for measuring the effectiveness of emulsifiers. One consists of measurements of such physical properties of the system as surface or interfacial tensions (*7, 19*), electrical charges (by electrophoresis), potential difference between disperse phase and dispersion medium, thickness of the protective film, viscosity, or specific gravity (*2*). All these quantities are undoubtedly of some importance in determining stability, but it is doubtful whether a single measurement of any of these properties constitutes, of itself, a determination of the stability of the emulsion. For example, the interfacial tension is probably an index of the ease of emulsification but is unreliable as a measure of the stability of the resulting emulsion (*20, 21*).

Another method of measuring effectiveness is the determination of either the maximum volume of disperse phase emulsified by a definite amount of emulsifier (*4*) or the smallest amount of emulsifier necessary to emulsify given volumes of different liquids (*8, 22*). These two methods measure the relative amounts of stabilizer and disperse phase necessary to produce an emulsion but do not measure its stability.

A third method consists of measuring the amount of salt necessary to separate the two phases (*16*). This method is not completely satisfactory as a measure of stability for different types of emulsions because the relative importance of electrical charge in determining stability may differ in various cases. Thus, emulsions in which stability is primarily due to the electrical charge on the droplets would probably appear less stable by this test than emulsions stabilized by a neutral film, although by other methods the two might appear equally stable.

A fourth method consists of the determination of the time required for a light to glow when the emulsion is placed between electrodes in series with a light bulb (*18*). This method also overemphasizes electrical factors contributing to stability and is applicable only to certain types of emulsions.

A fifth method is the determination of the time required for noticeable separation of phases on standing (*23*); and a sixth consists of measuring the variation with time of the droplet-size frequency (*6*) or interfacial area (*10*). A single size-frequency measurement is not sufficient because, as King (*9*) has pointed out, it appears that emulsion stability is not, in general, a function of the degree of dispersion. In utilizing this sixth method one should make sure that the change in droplet-size frequency actually represents phase separation and not merely an increase of droplet size to a larger, more stable form.

Other methods utilized for evaluating emulsions involve determining the oil content of horizontal layers of day-old emulsions (which involves creaming as well as breaking, *17*), observing the shapes of droplets of fungicidal emulsions dropped into water (*5*), measuring the toxicity to guinea pigs of dispersed bacterial toxin (which also involves the degree of dispersion of the emulsion and a possible detoxifying effect of the emulsifier, *15*), and the rate of saponification of oils (*13, 14*).

The methods that are fairly rapid are, in general, probably not valid measures of stability, whereas the more reliable methods, involving measurement of the separation of internal phase, are somewhat tedious and require measurements over a period of months and perhaps even years. Thus Berkman (*1*) mentions emulsions which show no signs of separation in five years. There is,

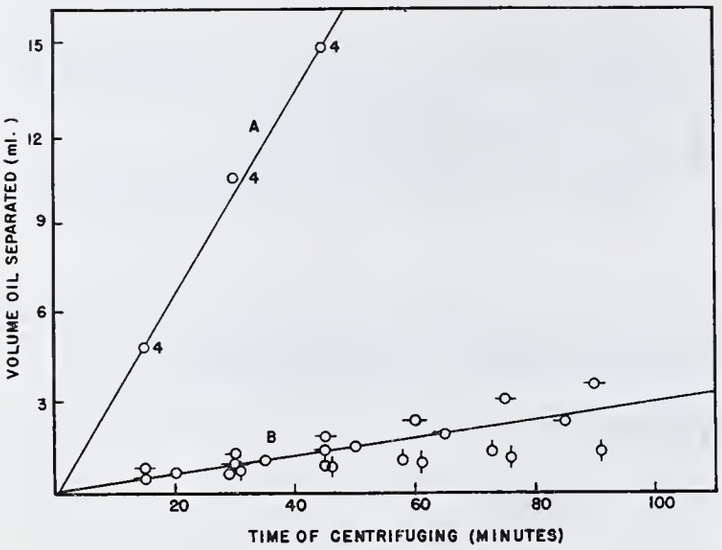

FIGURE 1. RATE OF SEPARATION OF BUTYL PHTHALATE FROM AQUEOUS EMULSIONS STABILIZED BY SODIUM ALEURITATE

Curve	Soap	Rate, Ml./Min.	K
A	0.1% sodium aleuritate (4 days old)	0.33	3.0
B	0.1% sodium aleuritate (fresh soln.)	0.030	33.0

The circles with different markings represent different runs on sodium aleuritate solutions varying in age by a few hours. Speed of centrifuging, 3000 r. p. m.

therefore, a definite need for reliable quantitative methods of measuring the stability of emulsions capable of giving results in a few hours. Since the rate of breaking of emulsions is the only reliable criterion of their stability, we have accelerated the breaking by centrifuging them in graduated tubes and have determined the rate of separation of internal phase at a constant speed of rotation. The reciprocal of the rate of phase separation is taken as a quantitative measure of the mechanical stability of the emulsion and is designated by K.

Experimental Method

The centrifuge used is a No. 1, Type SB International clinical centrifuge, equipped with horizontal heads carrying either four or eight 50-ml. tubes, a slide-wire resistance, and a Waltham tachometer reading up to 6000 r. p. m. The speed can be kept constant to about ±20 r. p. m. The distance from the center of rotation to the far end of the 9.5-cm. metal tubes used is 20.5 cm. The centrifugal force can be calculated from the formula

$$F = \frac{4\pi^2 n^2 mr}{3600 \times 980}$$

where F is the force, in multiples of the gravitational force, acting on a particle of mass m grams a distance of r centimeters from the axis of rotation at a speed of n revolutions per minute. The force acting on a particle in the center of the tube is 176, 1580, and 2280 times gravity at speeds of 1000, 3000, and 3600 r. p. m., respectively.

The emulsions were contained in graduated 40-ml. Pyrex, heavy-duty conical-bottom centrifuge tubes. During centrifuging the tubes were covered with a piece of sheet rubber held in place by a rubber band in order to minimize evaporation and aging effects on soap emulsions due to absorption of carbon dioxide from the air. The time of centrifuging was measured with an automatic electric timer equipped with a warning device. The volume of the dispersed phase which had broken from each emulsion was noted at successive intervals of 5 to 30 minutes and plotted as a function of time at a constant centrifuging speed. Care was taken in stopping the centrifuge to avoid convection currents which might otherwise have affected the results. Any temperature rise in the samples on prolonged centrifuging is so small as to be negligible.

Chemicals

The soaps of aleuritic (9,10,16-trihydroxypalmitic), palmitic, and stearic acids were made from the pure Eastman acids by neutralization in acetone solution with sodium ethylate for the sodium soaps, and with the corresponding hydroxide for ammonium and potassium palmitates. Sodium oleate was made from a technical oleic acid. Heavy metal soaps were made by precipitating them from hot solutions of the corresponding sodium soaps with a slight excess of the alkaline earth chloride. The precipitates were washed with hot water and dried in a vacuum desiccator.

The sodium palm-oil soap was made from an Ecuadorian palm oil (supplied by E. B. Kester, Western Regional Research Laboratory) in which the free fatty acids had an equivalent weight of 242. The saponin, cyclohexanol, lecithin, butyl phthalate, and castor oil were Eastman products. Aerosol OT, a trade name for the sodium salt of the dioctyl (2-ethylhexyl) ester of sulfosuccinic acid, is a pure wetting agent obtained from the American Cyanamid and Chemical Corporation. The benzene was the thiophene-free grade obtained from Eimer & Amend. The carbon tetrachloride, kerosene, tragacanth (U. S. P. No. 2), karaya (XXX grade), and acacia gums were commercial products. The pectin was a commercial citrus pectin with a jelly grade of 200.

Soap solutions were prepared immediately before using from freshly boiled distilled water. Accurately weighed amounts of solid soap were dissolved in sufficient water to give the desired volume.

Preparation of Emulsions

Emulsions were prepared by running the two liquids four or five times through a hand-operated aluminum emulsifier or homogenizer (Central Scientific, Catalog No. 70,180), in which a piston forces the liquids through six small slits between parallel plates of the 8-mm. nozzle, producing a rather effective shearing action. Except where otherwise stated, equal volumes of the organic liquid and of aqueous solutions of the stabilizing agent were emulsified. The concentrations of stabilizing agent given are based on the volume of aqueous solution, not on the total volume of emulsion. All experiments were carried out at room temperature.

For preliminary work on the method, emulsions were used in which the internal phase had a higher density than the dispersion medium, since volume readings at the bottom of the centrifuge tubes are easier to make and can be made more precisely. It was also thought that if the internal phase were heavier, the centrifugal force would accelerate breaking rather than creaming, thus making the results more significant. However, later work showed the method to be applicable even when the disperse phase is lighter than the dispersion medium. Dibutyl phthalate (density 1.0465) was chosen as the internal phase, since dye tests using methylene blue and Orange OT showed that it formed oil-in-water emulsions with the water-soluble soaps used as emulsifiers.

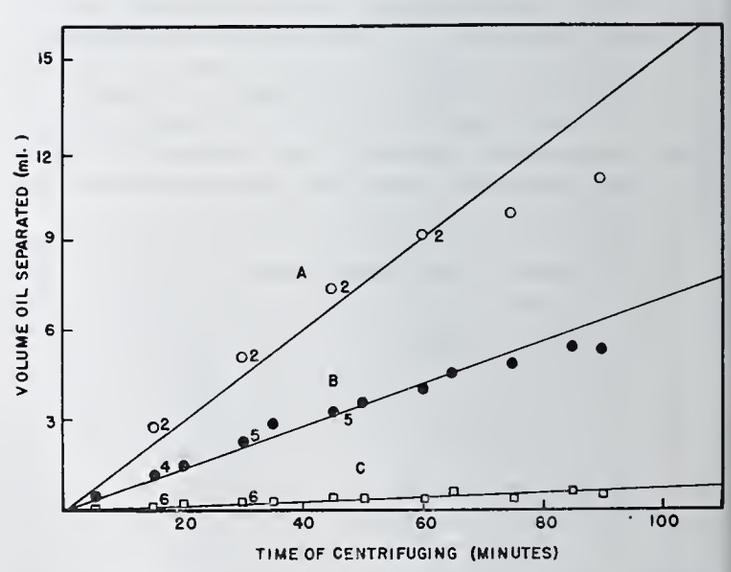

FIGURE 2. RATE OF SEPARATION OF BUTYL PHTHALATE FROM AQUEOUS EMULSIONS STABILIZED BY SAPONIN AND SODIUM PALM-OIL SOAP

Curve	Soap	Rate, Ml./Min.	K
A	0.1% saponin (4 days old)	0.150	6.0
B	0.1% saponin (fresh soln.)	0.070	14.6
C	0.1% sodium palm-oil soap	0.006	166.0

Speed of centrifuging = 3000 r. p. m.

Experimental Data and Discussion

Representative data obtained are shown in Figures 1 to 5, in which volume of oil or internal phase separated is plotted as a function of the time of centrifuging. The rate of separation is found to be practically constant until about 60 to 75 per cent of the internal phase has separated; above this region the rate decreases markedly, although the whole curve does not appear to be logarithmic. After visual inspection of the data, straight lines corresponding to the initial rate were drawn so as to give the best fit of experimental points. Freshly prepared emulsions containing unemulsified oil show separation at the same rate as the completely emulsified systems.

The formula for the rate of separation of phases proposed by Lederer (*11*) does not apply either to the data of this investigation or to all the previous data of Cheesman and King (*3*) obtained by measuring the rate of separation on standing.

Since the rate-of-separation curves appear to be similar in type for the emulsions studied, it is sufficient for practical purposes to take the initial rate of separation of disperse phase as an index to the mechanical stability of the emulsion. Logically, it is preferable to call this a measure of the mechanical instability and to take the reciprocal of this rate as a measure of the mechanical stability. For rapid comparative studies only the relative amounts of internal phase separated after a constant arbitrary time of centrifuging need be measured.

Aging Effects

Emulsion stabilized by saponins and soaps show pronounced aging effects after being allowed to stand in a beaker covered with a watch glass, even though thoroughly re-emulsified by the previously used technique immediately before centrifuging. The extent of aging varies with the stabilizing agent. The rate of separation of dibutyl phthalate at a speed of 3000 r. p. m. from emulsions stabilized by 0.1 per cent sodium aleuritate increases tenfold (from 0.030 to 0.33 ml. per minute) in 4 days, whereas from those stabilized with 0.1 per cent saponin the rate little more than doubled (from 0.070 to 0.150 ml. per minute) in the same time. This rapid aging of sodium aleuritate solutions accounts for the slightly different rates obtained in different runs on emulsions from supposedly fresh solutions whose age actually varied by as much as 6 or 8 hours. Different points from the same run form approximately straight lines when plotted as in Figure 1. The aging effect is probably due at least partly to absorption of carbon dioxide and subsequent hydrolysis of the soap. The acid soaps formed are evidently much less effective stabilizing agents. Saponin solutions also decompose on standing. Such pronounced aging effects were not obtained with emulsifiers which are stable when exposed to air, nor would it be expected that they would be obtained with soap emulsions kept in closed vessels free from carbon dioxide.

Reproducibility of Method

As an indication of the reproducibility of the centrifugal method in its present state, the values from five independent runs on butyl phthalate—0.1 per cent sodium aleuritate emulsions (aged for 4 days and then re-emulsified)—are presented. The amounts of butyl phthalate separated at the end of various periods at 3000 r. p. m. were as follows:

After 15 Min.	After 30 Min.	After 45 Min.	After 60 Min.
Ml.	*Ml.*	*Ml.*	*Ml.*
4.0	11.0	16.0	17.1
5.2	10.4	14.9	17.1
5.1	10.2	14.4	17.1
4.9	11.1	14.5	
4.6	10.4		
Av. 4.8	Av. 10.6	Av. 14.9	Av. 17.1

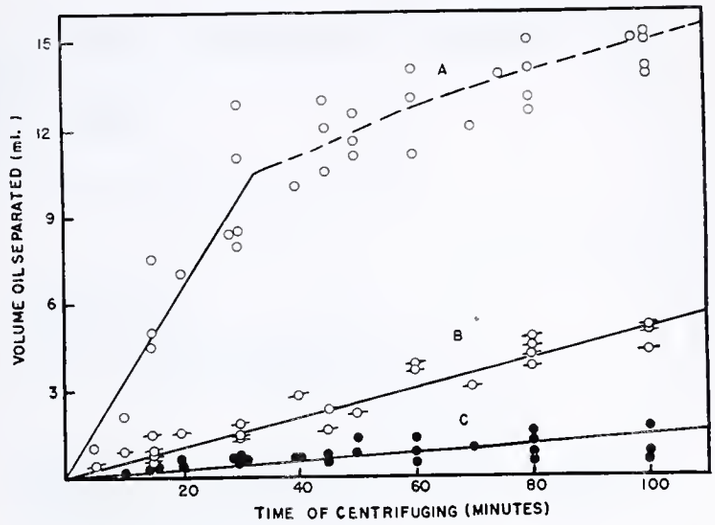

Figure 3. Rate of Separation of Butyl Phthalate from Aqueous Emulsions Stabilized by Pectin and Vegetable Gums

Curve	Stabilizer	Rate, Ml./Min.	*K*
A	1% karaya gum	0.31	3.2
B	0.3% tragacanth	0.051	20.0
C	1% pectin	0.014	71.0

Speed of centrifuging = 3600 r. p. m.

The average values are used in Figure 1. Data for the other solutions and figures were obtained from three to six independent runs.

Comparison of Emulsifiers

Sodium palm-oil soap forms more stable emulsions with dibutyl phthalate than either saponin or sodium aleuritate does when compared at 0.1 per cent. Under the same centrifugal force, palm-oil-soap–stabilized emulsions show phase separation at about one fifth of the rate of fresh sodium aleuritate emulsions and about one tenth of the rate of fresh saponin emulsions. Butyl phthalate emulsions stabilized by 0.1 per cent vegetable gums are of larger droplet size and break much more rapidly than those stabilized by soaps. Lecithin emulsions are very stable and behave more like soap-stabilized emulsions than those stabilized with pectin or vegetable gums.

Figure 3 again shows the apparent constancy of the initial rate of separation of butyl phthalate from pectin, karaya, and tragacanth emulsions and the rapid decrease in rate after about 60 per cent of the karaya emulsion has broken. Karaya-stabilized emulsions break unevenly and as a result the error of reading is increased. This accounts for the somewhat greater spread of experimental points for karaya. Emulsions stabilized by gum acacia break even more rapidly than karaya emulsions. The order of decreasing stability of butyl phthalate emulsions is: tragacanth > pectin > karaya. Butyl phthalate emulsions stabilized by gum acacia are less stable than those stabilized by karaya.

Emulsions stabilized by 0.3 per cent lecithin appear to lose butyl phthalate at the constant initial rate of 0.057 ml. per minute (Figure 4). Lecithin is about equivalent to tragacanth as an emulsifier for dibutyl phthalate, although perhaps not quite so good.

Emulsions with Less Dense Internal Phase

In order to see whether the centrifugal method can be used when the droplets are lighter than the dispersion medium, equal volumes of 0.1 per cent Aerosol OT solutions and the following organic liquids were emulsified and centrifuged at 3000 r. p. m.: benzene (density 0.879), cyclohexanol (density 0.945), castor

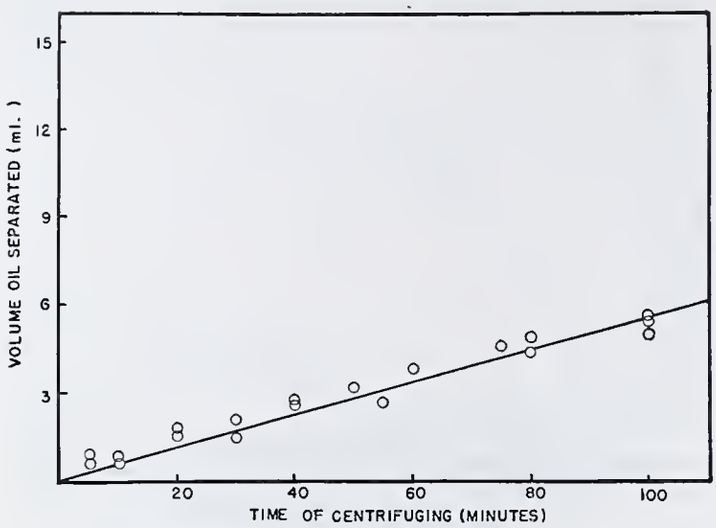

FIGURE 4. RATE OF SEPARATION OF BUTYL PHTHALATE FROM AN AQUEOUS EMULSION STABILIZED BY 0.3 PER CENT LECITHIN

Rate = 0.057 ml./minute, K = 18.
Speed of centrifuging = 3600 r. p. m

oil (density 0.96), and mineral oil (density 0.85). Dye tests using Orange OT and methylene blue showed the emulsions to be of the oil-in-water type. On centrifuging, the liquid in the droplets separated at the top of the emulsion at the rate of 0.042, 0.069, and 0.31 ml. per minute for the cyclohexanol, benzene, and castor oil, respectively (Figure 5).

Castor oil, the density of which is nearest to that of water and therefore might be expected to separate more slowly than any of the others, separates the most rapidly. The fact that the separation rate of these emulsions is not determined only by the difference in densities of the phases is evidence that the centrifugal method measures emulsion stability. At any rate, the data show that the centrifugal method can successfully be used, even when the density of the disperse phase is less than that of the dispersion medium. However, care must be taken in interpreting comparative measurements obtained by this method on disperse phases of varying densities. Recently King (*9*) has presented evidence indicating that the stability on aging and resistance to salting out by potassium sulfate of aqueous emulsions with nonpolar liquids increased with decreasing difference between the densities of the two phases. The centrifugal method here described would probably yield the same result.

In addition to the liquids mentioned above the method has been used on aqueous soap-stabilized emulsions of cresol, aniline, toluene, kerosene, carbon tetrachloride, and mixtures of the last two. Experiments on water-in-oil emulsions of benzene and kerosene stabilized by heavy-metal soaps indicate that the method is applicable to these systems.

Comparison with Previous Investigations

After centrifuging at 3600 r. p. m. for 100 minutes the volumes of kerosene separated from a 50–50 emulsion stabilized by 0.005 N potassium, sodium, and ammonium palmitates were 1.3, 1.5, and 1.7 ml., respectively. This is the same order found by King and Mukherjee (*10*) for these same soaps at 0.001 N by the method of rate of decrease of interfacial area. Tests on mineral-oil emulsions stabilized by 1.66 per cent carbohydrate colloids indicated the order of increasing mechanical stability to be tragacanth $<<$ acacia $<$ karaya $\sim$ pectin. This is in qualitative agreement with the data of Lotzkar and Maclay (*12*) on these identical emulsions obtained by measuring the rate of decrease of interfacial area.

While the agreement between the rate of decrease of interfacial area and the centrifugal methods of measuring emulsion stability appears satisfactory, final proof of the value of either method for many practical purposes must await the comparison of data obtained by these methods with data on identical systems obtained by measuring the rate of separation on standing.

Preliminary tests have shown that increasing the centrifuge speed increases the rate of phase separation. It is possible that more experiments might show the rate of separation of the internal phases to be directly proportional to the applied centrifugal force. In that case, the reciprocal of the rate of separation divided by the average centrifugal force would be a preferable unit.

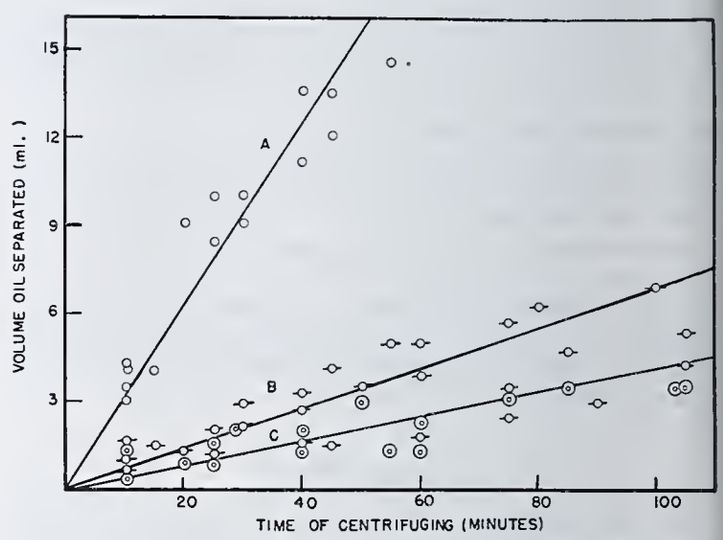

FIGURE 5. RATE OF INTERNAL PHASE SEPARATION FROM 0.1 PER CENT AEROSOL OT EMULSIONS

Curve	Organic Liquid	Rate, Ml./Min.	K
A	Castor oil	0.31	3.2
B	Benzene	0.069	14.5
C	Cyclohexanol	0.042	24.0

The double circles are points for cyclohexanol.
Speed of centrifuging = 3000 r. p. m.

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Rapid Iodometric Determination of Iron in Iron Phytate

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A direct titration method makes it possible to determine iron in ferric phytate in about 20 minutes, whereas other methods adaptable to the product require several hours. The results obtained are highly reproducible. The method is more accurate than methods which involve considerable manipulation and possible loss.

FERRIC phytate has been proposed as a source of iron for the nutritional fortification of grain and cereal products. It is a buff-colored compound, insoluble in water and dilute acids, which does not accelerate the development of rancidity in foodstuffs. The iron is available to the animal organism and, since phytic acid is a natural constituent of cereals, no foreign matter is introduced (*1*).

Since phytic acid forms iron salts of varying iron content, its analysis before incorporation into food products seems highly desirable. The usual analytical procedures that might be applied to this compound are lengthy and offer considerable difficulty. Ferric phytate, however, exhibits certain properties that allow a much more rapid determination of iron. Posternak (*5*) has shown that iron can be quantitatively split from the ferric phytate by strong alkali, producing the insoluble ferric hydroxide and the soluble alkaline phytate. By heating a sample of ferric phytate in a strong sodium hydroxide solution, filtering, washing the soluble sodium phytate from the ferric hydroxide, then dissolving the latter in hydrochloric acid, the iron content can be determined by the usual Zimmerman-Reinhardt method. This procedure, though satisfactory, is also tedious. The authors found that ferric phytate is very soluble in concentrated hydrochloric acid, and that under these conditions the ferric ion is readily available. It is sufficient, therefore, to add a potassium iodide solution of the proper concentration to the strongly acid solution of the ferric phytate and, after dilution, to titrate the liberated iodine with thiosulfate. Two samples of ferric phytate have been analyzed for iron in this manner with very gratifying results. Comparisons with determinations made by the Zimmerman-Reinhardt method are satisfactory.

The iodometric determination of iron is usually considered unsatisfactory, as the reversibility of the reaction results in the almost immediate return of the blue starch-iodine color. According to Kolthoff and Furman (*2*) the equilibrium expression for the iodometric titration of the ferric ion:

$$2Fe^{+++} + 2I^- \rightleftharpoons 2Fe^{++} + I_2$$

is given as

$$\frac{[Fe^{++}][I_2]^{1/2}}{[Fe^{+++}][I^-]} = 21.1$$

In order to obtain quantitative results, it is recommended that the ferric ion be present as the salt of a strong mineral acid; that the solution be strongly acid, pH 1; and that an excess of the iodide be present. However, when ferric phytate is titrated iodometrically, in no case does the color return in less than 60 minutes, and 2 to 3 hours usually elapse before there is a recurrence. Thus the reduction of the ferric ion is of a more permanent nature. Undoubtedly a soluble, but little dissociated, ferrous potassium salt of phytic acid is formed which is not easily oxidized to the ferric state, as is the case when an inorganic ferric salt reacts with potassium iodide. This reaction may be represented as follows:

$$(Fe^{+++})_x\text{phytate} + 3x(HCl) \rightleftharpoons x(FeCl_3) + \text{phytic acid} \quad (1)$$

$$x(Fe^{+++}) + x(KI) + \text{phytic acid} \rightleftharpoons (Fe^{++}K)_x\text{phytate} + x(I) \quad (2)$$

Another (less probable) possibility is that the phytic acid formed in Equation 1 acts as an inhibitor of the oxidation of the ferrous chloride formed when the potassium iodide reacts with the ferric chloride.

The addition of phytic acid when iron is determined iodometrically in ferric ammonium sulfate delays the return of the blue starch-iodine color, just as in the case of the ferric phytate titrations. Although not yet completely worked out, it appears that the use of phytic acid could be adapted to the iodometric titration of other iron compounds.

Experimental

PREPARATION OF IRON PHYTATE SAMPLES. A solution of phytic acid was prepared from wheat bran by the method of Morrow and Sandstrom (*4*). Samples of iron phytate were prepared from this solution by precipitating with ferric chloride at pH 4 to 5, washing with dilute acid and finally with water, and drying in vacuum over phosphorus pentoxide.

Iron was determined in 1-gram samples by the accepted Zimmerman-Reinhardt method (*3*) after digestion with sulfuric and perchloric acids (Table I).

DIRECT TITRATION METHOD. Samples of 0.5 to 1.5 grams were transferred to 250-ml. iodine flasks, 10 ml. of hydrochloric acid were added, and the flasks were gently rotated to effect complete solution. Then 25 ml. of a 16 per cent potassium iodide solution were added, and the flask was quickly stoppered and twirled several times to ensure thorough mixing. The mixture was allowed to stand 5 minutes, 100 ml. of water were added, and the liberated iodine was titrated with 0.1 *N* sodium thiosulfate using starch indicator. The titrations were corrected for blanks run under identical conditions (Table I).

CALCULATION. 1 ml. of 0.1 *N* sodium thiosulfate is equivalent to 0.05585 gram of iron.

TABLE I. DETERMINATION OF IRON

No. of Determinations	Sample Weight	Iron Found: Direct titration	Iron Found: Zimmerman-Reinhardt
	Grams	%	%
		Sample A	
3	0.50	Max. 16.88 Min. 16.82 Av. 16.86	...
15	0.70	Max. 16.88 Min. 16.82 Av. 16.83	...
19	1.00	Max. 16.88 Min. 16.77 Av. 16.83	...
2	1.50	Max. 16.79 Min. 16.79 Av. 16.79	...
6	1.00	...	Max. 16.82 Min. 16.63 Av. 16.70
Av. of all determinations		16.82	16.70
		Sample B	
13	1.00	Max. 11.57 Min. 11.45 Av. 11.49	...
3	1.00	...	Max. 11.32 Min. 11.21 Av. 11.26

[1] Present address, Chicago Quartermaster Depot, Q.M.C. Subsistence Research Laboratory, Chicago, Ill.

Table II. Blanks Obtained under Varying Conditions

No. of Determinations	HCl	KI per 25 Ml. of Solution	Time of Standing	0.1 N $Na_2S_2O_3$, Average
	Ml.	*Grams*	*Min.*	*Ml.*
3	10	5.0	15	0.25
3	10	2.5	15	0.17
6	10	4.0	5	0.14
2	20	4.0	5	0.23
3	10	7.5	15	0.29

Discussion

Several factors must be carefully controlled. It is advisable to use samples of 0.7 to 1.00 gram, as then the volume of the thiosulfate used is well within the desirable titration range. The ratio of the sample weight in grams to the volume of hydrochloric acid in milliliters must be kept between 1 to 7 and 1 to 15 to ensure complete solution and yet avoid too great an excess of acid. The ratio of the volumes of the hydrochloric acid and potassium iodide solution must be kept between 1 to 2 and 1 to 3 to prevent the precipitation of the ferric phytate and yet maintain as low an acid concentration as possible. There is a blank liberation of iodine which depends upon the amount of potassium iodide and hydrochloric acid and the time of standing (Table II). Varying these three factors within the limits set up herein does not affect the determination, provided a correction is made for a blank run under identical conditions.

It is suggested that as a routine procedure the volume of the hydrochloric acid and potassium iodide solution be accurately measured by pipet and the time of standing be recorded on a stopwatch. It will then be necessary to run only one set of blanks for each batch of hydrochloric acid and potassium iodide for different times of standing. A 1 to 50 dilution of the thiosulfate in the blank titration will give more reliable values. If the blank titration is plotted against the time of standing, the correction to be applied to any determination can be read off the graph.

The results by the Zimmerman-Reinhardt method are slightly lower (0.1 to 0.2 per cent) than those by the direct titration method, which can undoubtedly be attributed to incomplete digestion and solution.

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Quantitative Determination of Sulfanilamide and Sulfathiazole in Mixtures

Spectrophotometric Method

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THE wide use of the sulfa drugs has given rise to considerable interest in their analytical reactions. Their assay, differentiation, and identification have been the subject of a recent article by Calamari, Hubata, and Roth (*3*). The principal chemical reaction employed for the quantitative estimation of those substances involves the diazotization of the aryl amino group. By coupling the diazonium salt and production of an azo dye the compound may be estimated colorimetrically and the sensitivity greatly increased (*5*). Anderson (*1*) has called attention to the fact that free sulfanilamide and acetylated sulfanilamide have different absorption characteristics in the range 310 to 360 mμ and has devised a unique fluorometric method for the estimation of each.

A problem which was presented to the authors was the determination of sulfanilamide and sulfathiazole when present together in a mixture. It seemed probable that these compounds would show markedly different absorption characteristics in the ultraviolet range and that the determination of each could be accomplished by a physical method without separation of the constituents. In 1930 Barnard and MacMichael (*2*) demonstrated that a color system of two components may be quantitatively analyzed, even if both components show absorption at the selected wave length, provided the degree of absorption is sufficiently different and the sample represents a definite total amount of the two constituents only. Knudson, Meloche, and Juday (*4*) have pointed out an extension of the general principle and have indicated how the method may be used for more than two components. It is necessary to determine the extinction coefficients of each pure component at selected wave lengths which give the widest differences in absorption of one from the other components. The extinction value of the mixture is then determined at the selected wave lengths and the proportions of the various constituents are calculated from the data by the solution of a series of simultaneous equations.

It was the object of this study to develop a method for the analysis of the sulfa drug mixture based upon this principle.

Experimental

The instrument employed in this work was a Bausch & Lomb medium quartz spectrograph. The slit was adjusted to a width of 0.06 mm. A Wood's type hydrogen discharge tube, which gives a continuous spectrum of fairly uniform intensity over the desired wave-length range, served as the source of illumination. The source was placed with the exit window at a distance of 10 cm. from the slit. A cell of 1-cm. length with quartz windows was used to hold the liquids during their examination. Separate exposures of the solvent and the solutions were taken for a period

Table I. Determination of Sulfathiazole and Sulfanilamide in Mixtures by Spectrophotometric Examination at 2600 and 2875 Å.

(Values expressed in mg. of constituent per liter)

Mixture	Sulfanilamide Added	Sulfanilamide Found	Sulfanilamide Deviation	Sulfathiazole Added	Sulfathiazole Found	Sulfathiazole Deviation
1	2.50	2.55	+0.05	1.25	1.17	−0.08
2	8.33	8.37	+0.04	1.60	1.42	−0.18
3	10.00	9.70	−0.30	2.00	1.97	−0.03
4	2.00	1.97	−0.03	10.00	10.40	+0.40
5	5.00	5.20	+0.20	5.00	5.09	+0.09
6	7.00	6.95	−0.05	3.00	3.11	+0.11
7	3.00	3.30	+0.30	7.00	7.50	+0.50
8	1.25	1.29	+0.04	2.50	2.70	+0.20

[1] Present address, Standard Oil Co., Richmond, Calif.

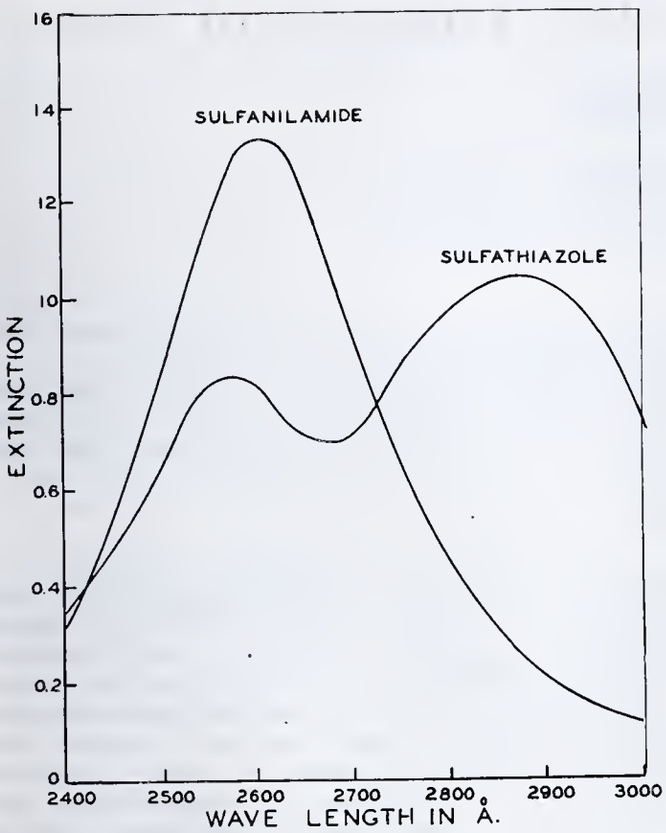

FIGURE 1. ABSORPTION CURVES

of 2 minutes each. The spectra were recorded on Eastman Polychromatic plates. Each plate was calibrated by taking a series of separate successive exposures in which the time interval was varied in a regular manner as follows: 4, 8, 16, 32, 64, and 128 seconds. The plates were developed for 6 minutes in Eastman x-ray developer at 18° C., then fixed, washed, and dried. After drying, the densities of the spectrograms at selected wavelength intervals were determined with a Leeds & Northrup recording microphotometer. A family of plate calibration curves for the selected wave lengths was then constructed and by reference to the appropriate curve the relative intensity values for the pure solvent and the solution were found and from these the extinction value for the solution was calculated.

Figure 1 shows the absorption curves for pure sulfathiazole and pure sulfanilamide in 95 per cent ethanol solutions. The sulfanilamide solution shows an absorption peak at 2610 Å. and the sulfathiazole solution has two absorption peaks, one at 2580 Å. and one at 2875 Å. The sulfanilamide has a slight absorption at 2875 Å. also.

The absorption values at different concentrations of each compound were determined and the results are indicated in Figure 2. The solutions of both compounds obey the Beer-Lambert law at both 2600 and 2875 Å. Therefore, a quantitative estimation of each of the constituents is possible in a mixture of the two.

The data necessary for making a calculation of the quantity of each are given below:

E'_a = extinction for 1 gram per liter of sulfathiazole at 2600 Å.
E'_b = extinction for 1 gram per liter of sulfanilamide at 2600 Å.
E''_a = extinction for 1 gram per liter of sulfathiazole at 2875 Å.
E''_b = extinction for 1 gram per liter of sulfanilamide at 2875 Å.
E'_m = extinction for the mixture at 2600 A.
E''_m = extinction for the mixture at 2875 Å.

If x = grams of sulfathiazole per liter
y = grams of sulfanilamide per liter

Then $xE'_a + yE'_b = E'_m$
$xE''_a + yE''_b = E''_m$

From a solution of these equations the number of grams of each constituent in the solution is determined. The results of a group of analyses are given in Table I.

Discussion

An examination of Table I shows the order of agreement which may be expected in a series of analyses in which the ratios and total amount of the two constituents are varied. The procedure employed involved the assumption of a source of illumination of constant intensity. This assumption was checked by making a series of exposures of the source in which the time was constant for each. The variation was within the range of other experimental errors.

It is obvious that if the observations were made with an instrument which did not require the photographic process—for example, the photoelectric spectrophotometer adapted to the ultraviolet range—the errors which are inherent in the photographic method might be eliminated and the process of analysis greatly simplified. It is possible that solutions of higher concentration might be handled with such equipment and the accuracy of the method improved.

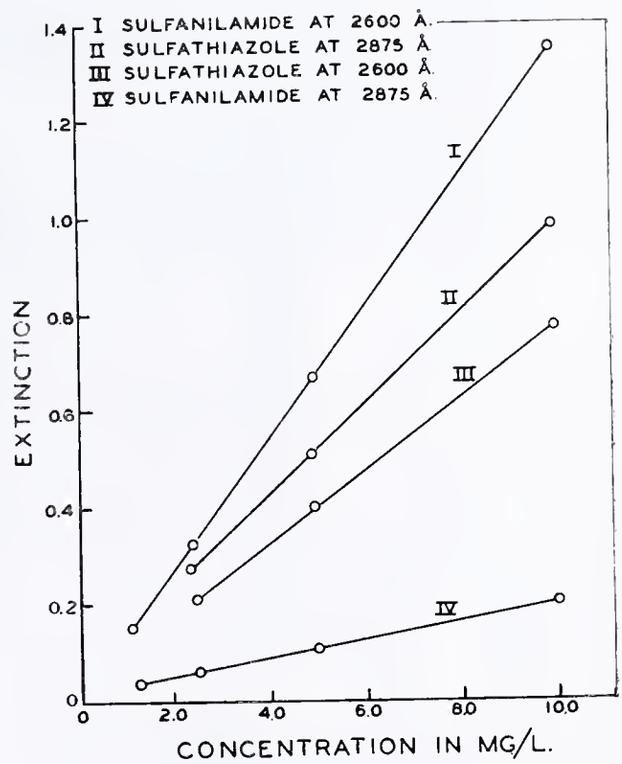

FIGURE 2. ABSORPTION VALUES

Acknowledgment

The authors wish to express their indebtedness to L. A. Hall The Griffith Laboratories, Chicago, Ill., at whose suggestion the problem was undertaken and from whom the samples employed in this study were obtained.

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Modified Diffusion Pump for Analysis of Gases in Metals

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IN RECENT years the effect of gases in metals on metal properties and the analyses of these gases have received increasing attention. The most direct methods for such analyses consist in heating or fusing the metal in a high vacuum and removing the evolved gases with the aid of a diffusion pump.

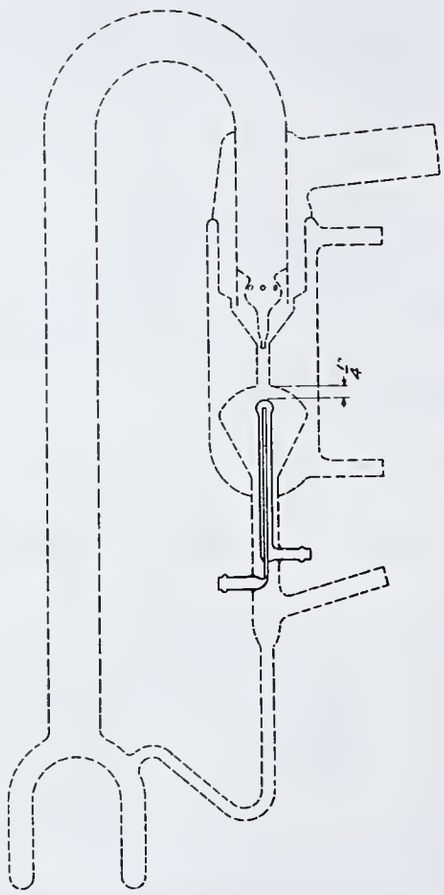

FIGURE 1. MODIFIED DIFFUSION PUMP

In certain schemes (*1*, *2*, *3*, *5*) employing a volumetric method of measuring gas quantities, the pump acts as a cutoff or valve to prevent return of evolved gases to the metal sample, and the outlet portion of the pump serves as part of the known volume which the gas occupies. It is usually assumed that the cutoff action of the mercury jet is sharp, and, consequently, that the volume of the system is constant for any gas pressure which is less than the critical back pressure under which the diffusion pump operates. The authors have observed, however, that the portion of the determined volume within the diffusion pump is to some extent dependent on gas pressure, and is, moreover, sensitive to the distillation rate of the mercury. The maximum variations observed were of the order of 35 ml. with major differences occurring in the low-pressure region (0 to 0.04 mm. of mercury). This maximum discrepancy may not be important if the total volume for gas confinement is of the order of liters, but in a system with a total volume in one case of 200 ml., the error introduced from this source could not be neglected.

It is possible to avoid an error of this kind by use of a Toepler pump to transfer all evolved gases into a portion of the system with known volume. The Toepler pump, however, adds complexity to any gas analysis system and increases the time required to complete the analysis so that the simpler system is preferred, particularly for industrial use.

In their experiments the authors employed a two-stage mercury diffusion pump of design illustrated by the dashed outline of Figure 1. The volume of the system, part of which included the diffusion-pump outlet, was determined by admitting nitrogen at known pressure, entrapping a portion of this gas in the McLeod gage, evacuating the system, then expanding the known volume of gas into the system and again measuring the pressure. From these two pressures and one volume, the volume of the system could be calculated. If the pressure of the admitted gas in check determinations was higher than for the previous runs, the calculated volume increased. In addition, if the mercury distillation rate was increased, the calculated volume decreased. These facts were checked by replacing the diffusion pump with another of the same general design and noting the same behavior.

The effect is not due to leakage past the mercury jet. Langmuir has shown (*4*) that pumps of this type will maintain a high vacuum against pressures greater than those used in the authors' determinations.

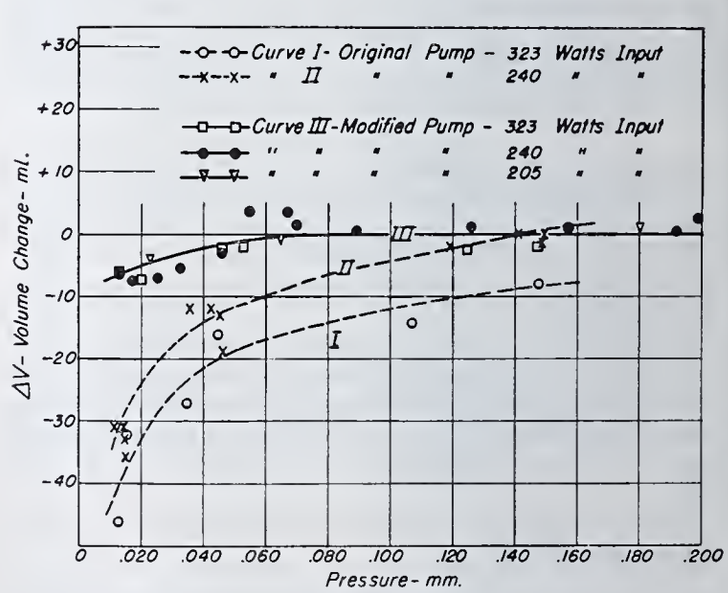

FIGURE 2. CHANGE OF CALCULATED VOLUME WITH PRESSURE AT VARIOUS DISTILLATION RATES

Apparently the change in volume with gas pressure is caused by the long mean-free-path mercury atoms traveling undeflected into lower portions of the pump condenser. They transform from the vapor phase at different levels, depending on the velocity of the mercury atoms and on the mean pressure of the

gas into which they are projected. Langmuir suggested to the authors that if the mercury vapor stream were deflected and condensed at a definite point below the jet and inside the condenser, any observed volume change should be diminished. They are indebted to him for the suggestion of sealing an additional water-cooled tube into the condenser to accomplish this purpose, as shown by the heavy outlined portion of Figure 1. This modification definitely improved the reproducibility of calculated volumes at various pressures without impairing the pumping performance. Furthermore, the measured volume was now insensitive to mercury distillation rates.

The comparative results are shown in Figure 2. Curves I and II represent differences in calculated volumes as a function of gas pressure employing the conventional design of pump. Curve I is for the higher mercury distillation rate corresponding to 323 watts supplied to the electric resistance heater; and curve II, the lower distillation rate, corresponding to 240 watts. Curve III is for a similar system employing the modified diffusion pump. Determined volumes of the system are better reproducible and the maximum deviation of the mean volume as the pressure is lowered to 0.01 mm. of mercury is less than 10 ml. Of further importance is the fact that the data at three distillation rates practically coincide.

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Modified Amino Nitrogen Apparatus for Insoluble Proteins

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An auxiliary reaction chamber for use with a manometric Van Slyke apparatus is described. The apparatus enables one to determine easily the rate of liberation of amino nitrogen from insoluble proteins and thereby to determine their free amino nitrogen content.

SINCE the chemical modification of proteins frequently involves the free amino groups, it is advantageous to be able to determine the free amino nitrogen remaining after chemical treatment. Most of the protein derivatives are insoluble under the conditions of analysis, and some have a rather low free amino nitrogen content. The limitations of the Van Slyke method (*7*) are well known and have been summarized by Richardson (*5*). Its application to insoluble proteins has offered additional difficulties in the introduction of the sample into the reaction chamber and the selection of the time of reaction. Several means for solving these difficulties have been suggested (*1, 2, 3, 6*). Kanagy and Harris (*1*) have shown that an arbitrary reaction time may not give the correct value for free amino nitrogen in proteins and have utilized the rate of evolution of amino nitrogen to determine the free amino nitrogen present. Rutherford, Harris, and Smith (*6*) using a modified Shepherd gas analysis apparatus extended this method.

It seemed worth while to use this principle with an apparatus that was simpler and more adapted to routine determinations. With these requirements in mind an accessory reaction chamber for use with a standard manometric Van Slyke apparatus was designed which will permit introduction of large samples of solid material, removal of the gas evolved at stated intervals, and easy cleaning of the chamber at the end of the analysis. The calibrated Van Slyke extraction chamber is used only for measurement of the nitrogen evolved. The amino nitrogen content of several proteins and substituted proteins was determined with the aid of the auxiliary chamber.

Apparatus and Reagents

Figures 1 and 2 show the details of the auxiliary chamber and its relation to the Van Slyke apparatus. The chamber was constructed from a 34/45 standard taper ground-glass joint and mounted on a plate fastened to the extraction chamber carrier of the Van Slyke apparatus. The outer top half was constricted and sealed to a three-way 1-mm. bore capillary stopcock, *A*, fitted with a cup and a bent side arm similar to the stopcock of the Van Slyke extraction chamber. The inner bottom half was also constricted and sealed to a piece of 5-mm. bore heavy-walled tubing long enough to reach the pivotal point of the extraction chamber carrier. A yoke was fastened to each half of the chamber, and two connecting springs were provided to keep the chamber together. A 5-mm. bore stopcock, *B*, was fastened to the base of the Van Slyke apparatus and connected to the chamber with a piece of heavy-walled tubing. A 250-cc. leveling bulb was connected to *B* through a length of heavy-walled nitrometer tubing and hung in one of two rings on an adjacent ring-stand. Sufficient mercury to fill the chamber and one third of the leveling bulb was added. The rings were arranged in such a manner that with the bulb in the lower ring the mercury level was slightly above the 5-mm. bore tube and with the bulb in the upper ring the level was 10 cm. above the top of the chamber.

The reagents used are a solution of 40 grams of sodium nitrite in 50 cc. of water, glacial acetic acid, a saturated solution of sodium acetate, and capryl alcohol. Rutherford, Harris, and Smith (*6*) have shown that addition of sodium acetate decreases the size of the blanks. The standard manometric Van Slyke-Hempel pipet is filled with a solution of alkaline permanganate consisting of 50 grams of potassium permanganate and 25 grams of potassium hydroxide made up to 1 liter with water. The ground-glass joint of the chamber is well lubricated with a stiff vacuum grease before being assembled. The samples used are all ground to 80-mesh in a Wiley micromill, and the moisture content is determined at the time of analysis. The amount of protein used ranges from 0.5 to 1 gram.

Experimental Procedure

An excellent description of the care and use of the manometric Van Slyke apparatus has been given by Peters and Van Slyke (*4*). One familiar with the standard manometric amino nitrogen determination may proceed with the modified method as follows:

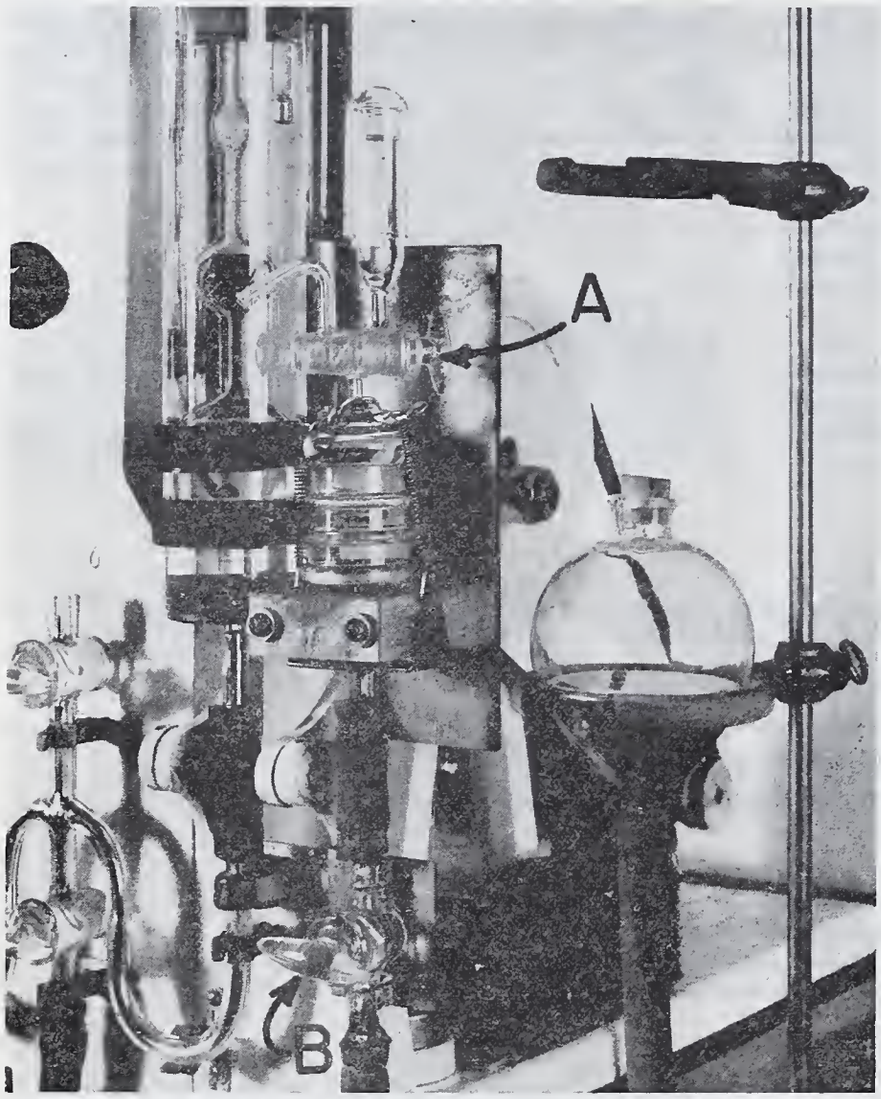

FIGURE 1. REACTION CHAMBER ATTACHED TO VAN SLYKE APPARATUS

Ten milliliters of water are admitted to the calibrated Van Slyke extraction chamber, the stopcock is closed, and the mercury is set at the 50-ml. mark. The top of the auxiliary chamber is removed, the mercury level set even with the bottom of the chamber, and stopcock *B* closed. The weighed sample of solid protein is introduced, followed by 2.0 ml. of glacial acetic acid, 2.0 ml. of saturated sodium acetate solution, and 5 drops of capryl alcohol. The chamber is assembled, and the bore of stopcock *A* and the capillary of the cup are filled with water. The side arm of *A* is connected with a high-vacuum pump, the pump started, and *A* turned to connect the pump with the chamber. The evacuation is continued for 2 minutes. The chamber is shaken throughout this period to facilitate removal of air from the mixture and prevent the formation of a cake. The shaking is then stopped, *A* is closed, and the vacuum line is disconnected.

Four milliliters of sodium nitrite solution are pipetted into the cup and run into the chamber by turning *A*, care being taken to admit no air. The cup and the bore of the stopcock are rinsed with 1 ml. of water, and the chamber is shaken 5 to 15 minutes, depending upon the amount of gas evolved. The shaking is then discontinued and *B* is opened to admit mercury to the chamber at an even rate. The leveling bulb is raised to the high ring to place the gas in the chamber under positive pressure. A few milliliters of water are placed in the cup, and the tip of a Hempel pipet is pressed in place. The Hempel stopcock is turned to connect the tip with the bulb, *B* is closed, and *A* is opened wide. *B* is then slowly opened, admitting mercury to the chamber and forcing the evolved gas into the Hempel pipet. *B* is closed when the reaction mixture is even with the bore of *A*. The Hempel stopcock is turned to the off position, the leveling bulb placed in the low ring, and *B* opened and then closed. The Hempel stopcock is turned to connect the cup with the tip, and *B* is opened slowly, allowing the gas in the Hempel capillary to be drawn back into the chamber along with enough water to seal the bore of *A*. *A* is closed, and the mercury level in the chamber is lowered until it is even with the bottom. *B* is then closed. The purified nitrogen in the Hempel pipet is transferred to the calibrated extraction chamber and measured in the usual manner.

Successive samples of gas are removed by the same procedure, the chamber being shaken for 10 minutes just prior to removal. The most effective control of liquids and gases is obtained through manipulation of the flow of the mercury through *B*.

Blank determinations were run in a similar manner, the protein being omitted. Selected time intervals generally were 10 minutes, 30 minutes, 1 hour, and each succeeding hour, the time being taken from the moment the sodium nitrite was added to the mixture. If the protein clings to the walls of the chamber when the mercury is lowered after a sample of gas has been removed, it may be dislodged by shaking the chamber while the mercury level is being lowered. The pressure readings obtained were corrected by subtracting a blank value for a similar time interval. The rate curves were obtained by plotting the total amino nitrogen liberated, in milligrams per gram of dry protein, against time. Duplicate values obtained from several proteins, modified proteins, arginine, and α-benzoylarginine amide by extrapolation of the secondary portion of the curve to zero time are given in Table I.

Discussion

Determinations were run in duplicate, and the rate curves for proteins and derivatives (Figure 3) plotted as previously described. The ratio of amino nitrogen to total nitrogen was plotted against time for arginine and α-benzoylarginine amide (Figure 4). The determinations were reproducible within the same limits of error as the manometric Van Slyke amino nitrogen determination (*4*). The values for the free amino nitrogen content of the proteins and derivatives in Table I were found by extrapolation of the secondary portion of the rate curve to zero time. This extrapolation is illustrated by the gelatin curve in Figure 3. The values can also be determined by calculation, using the straight-line equation:

$$y = mx + b$$

TABLE I. FREE AMINO NITROGEN CONTENT OF SOME PROTEINS AND DERIVATIVES DETERMINED AT 22–23° C.

Substance	Amino Nitrogen (Duplicate Determinations)	
	Mg./g. dry substance	
Wool	3.30	3.35
Silk (raw)	2.90	2.96
Gelatin (Eastman Kodak Co.)	8.70	8.74
Zein (Corn Products Refining Co.)	1.00	...
Casein	8.40	8.35
Benzoyl casein[a]	0.20	0.18
Desamino casein[a]	0.90	1.00
Arginine	80.2[b]	80.2
α-Benzoylarginine amide	0.0	0.0

[a] Prepared by J. L. Wood and A. P. Swain of this laboratory.
[b] Calculated, 80.4.

where y is the total milligrams of nitrogen per gram of dry protein obtained in time x, m the milligrams of nitrogen obtained per hour when the rate has become constant, and b the amount of free amino nitrogen initially present.

To achieve reproducible results and a rate curve capable of analysis by these methods, it is important to prevent the formation of aggregates in the chamber when the reagents are added. The material in the lumps so formed is not readily accessible to the action of the reagents and consequently evolves nitrogen slowly. The rate curve plotted from the values obtained in this instance has no definite change in slope and cannot be extrapolated in the usual manner to obtain an amino nitrogen value. This factor of aggregation is also of importance when the solution of a protein which is precipitated under the conditions of the analysis is admitted into the chamber. Thus the curd formed by an alkaline casein solution gave an indecisive rate curve similar to those obtained when aggregates of solid material were present. The use of a finely divided casein, well shaken to prevent the formation of lumps, obviated this difficulty. An examination of the curves obtained for the various proteins shows that one cannot arbitrarily select any reaction time that will be valid for different substances and conditions of analysis. Once the technique of handling this modified apparatus is acquired, the time required for the removal and measurement of a sample of gas—about 5 minutes—is approximately the same as in the standard Van Slyke determination. Since the reaction chamber is easily accessible and can be taken apart, it can be quickly and thoroughly cleaned at the end of each determination.

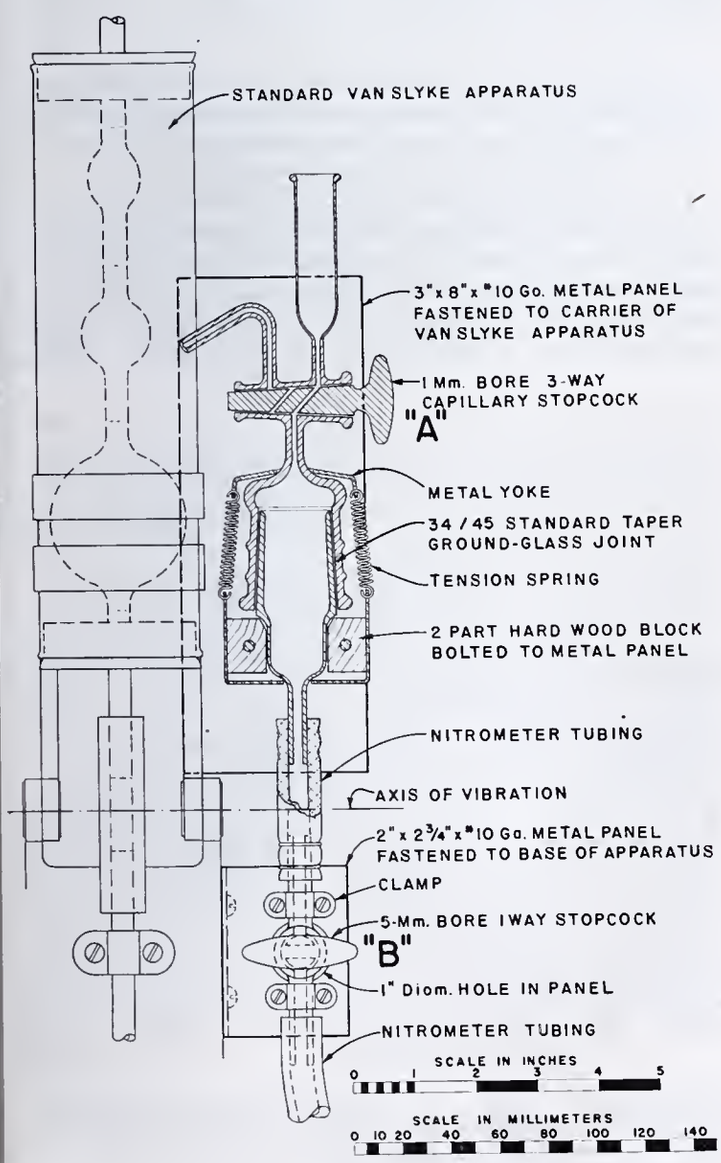

FIGURE 2. DIAGRAM OF REACTION CHAMBER

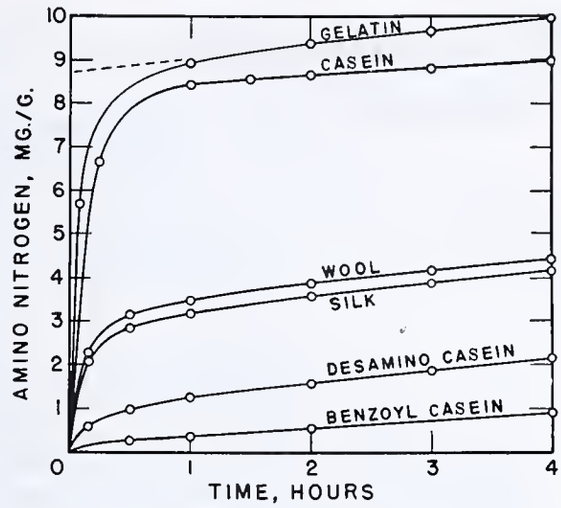

FIGURE 3. RATES OF EVOLUTION OF NITROGEN FROM VARIOUS PROTEINS

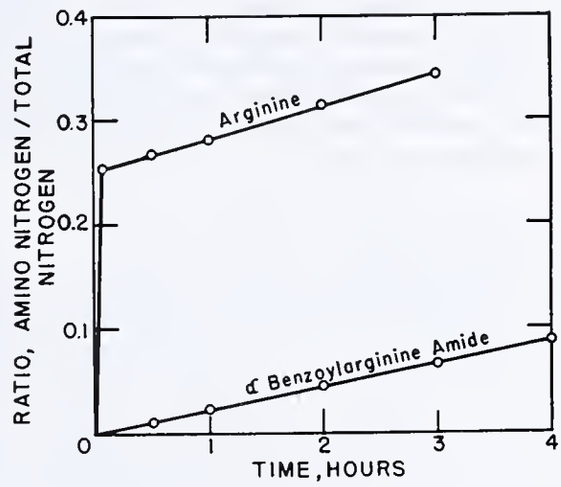

FIGURE 4. RATES OF EVOLUTION OF NITROGEN FROM ARGININE AND α-BENZOYLARGININE AMIDE

Summary

A simple apparatus for the routine determination of amino nitrogen of insoluble proteins and protein derivatives has been described. The rate of evolution of amino nitrogen from some insoluble proteins and protein derivatives was determined with this apparatus. The curves obtained by plotting the amino nitrogen content against time are similar to those obtained by Rutherford, Harris, and Smith (*6*) using a modified Shepherd gas analysis apparatus.

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Determination of Zinc in Biological Material

Photometric and Polarographic Methods Following Extraction with Di-beta-naphthylthiocarbazone

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Substitution of di-beta-naphthylthiocarbazone for dithizone in the extraction of zinc from solutions of ashed material and the incorporation of other improvements have enabled the development of colorimetric and polarographic methods possessing excellent sensitivity and specificity. The substitution of di-beta-naphthylthiocarbazone for dithizone has (1) eliminated partition losses occurring with dithizone in the pH range 8.3 to 10.5; (2) eliminated the loss of zinc which occurs otherwise when carbamate is used as complexing agent to remove interfering ions in the extraction solution; (3) permitted the derivation of colorimetric standardization curves which follow Beer's law over their entire range; and (4) made possible the addition of carbamate to the initial extraction step, a procedure which allows the analyst to fix the range and to extract zinc in sufficient purity to be determined either colorimetrically or polarographically.

Substitution of 0.2 *N* for 0.02 *N* hydrochloric acid permits stripping at least 60 micrograms of zinc from di-beta-naphthylthiocarbazone–carbamate-chloroform solution with a single 50-ml. portion of acid while eliminating interference by copper, cobalt, nickel, mercury, and silver.

The colorimetric method of estimation is recommended for determining very small quantities of zinc in samples containing no cadmium. When cadmium is present or when the quantities of zinc are greater than 0.05 mg. per 10 ml. of solution, the polarographic method is recommended.

A NUMBER of analytical methods for the determination of zinc in certain specific biological and related materials have been developed as an aid to the study of the physiological effects of zinc. Of these the most successful have been colorimetric procedures employing dithizone (*6, 7, 9, 10*) and a number based on polarographic (*8, 16, 18*) or spectrographic (*15, 17*) analysis. Spectrographic methods employing the most persistent zinc line at 2138 A. possess superior specificity but lack the sensitivity required for general biological work (*5*). Polarographic methods are satisfactory in respect to sensitivity, but high concentrations of many substances which are reduced at potentials below or near that of zinc may affect the polarographic wave of zinc. Colorimetric methods employing dithizone are extremely sensitive but require careful control of pH and prevention of the interferences of certain ions by the formation of nonreactive complexes. Thus far no reagent has been found which, when used with dithizone, will form satisfactory complexes with interfering ions without causing some loss of zinc. Through the use of these colorimetric methods, therefore, reproducible results can be obtained only by a rigid control of conditions to ensure uniform losses or contaminations.

In the methods described in this paper losses of zinc in extraction and most of the difficulties due to interfering ions are eliminated by employing a chloroform solution of di-beta-naphthylthiocarbazone and aqueous sodium diethyldithiocarbamate as the extraction agent. By the use of this extraction agent and the introduction of a number of other improvements, a sensitive and accurate mixed-color method has been developed. The initial extraction in the colorimetric procedure provides a means of concentrating the zinc and of removing it from large amounts of other reducible substances, so that it may be determined polarographically over a greater range of concentrations than is conveniently done by colorimetric means.

Di-beta-naphthylthiocarbazone

Di-beta-naphthylthiocarbazone (DN) is an analog of diphenylthiocarbazone (dithizone). Like dithizone it forms highly colored complexes with many metals. Its use as an analytical reagent was first suggested by Suprunovich (*19*), who claimed for it superior sensitivity. A small amount of this reagent was synthe-

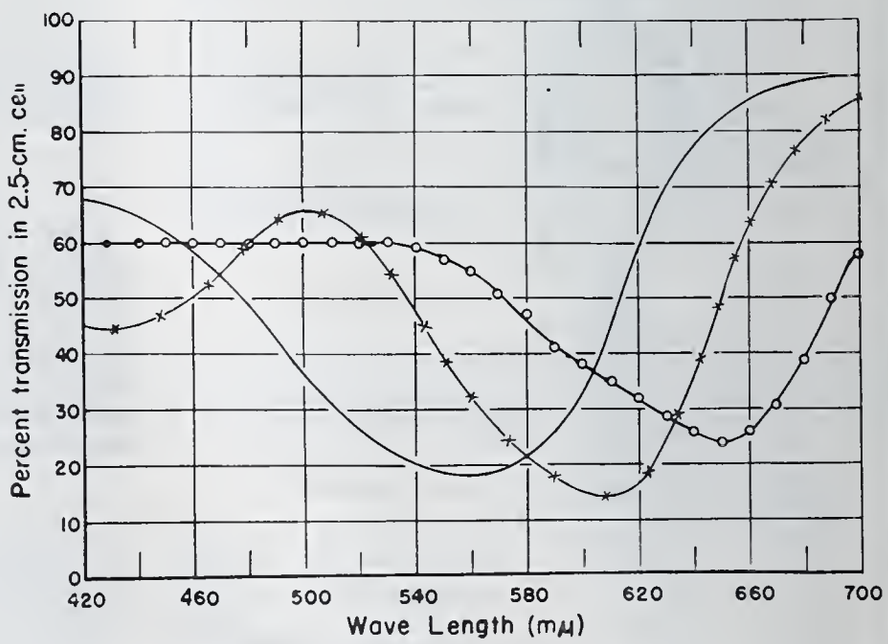

FIGURE 1. TRANSMITTANCY CURVES OF DITHIZONE, DI-BETA-NAPHTHYLTHIOCARBAZONE, AND DI-BETA-NAPHTHYLTHIOCARBAZONE COMPLEX OF ZINC IN CHLOROFORM

o. Pure DN in chloroform, 2 mg. per liter
x. Pure dithizone in chloroform, 2 mg. per liter
–. Pure DN totally converted to zinc complex

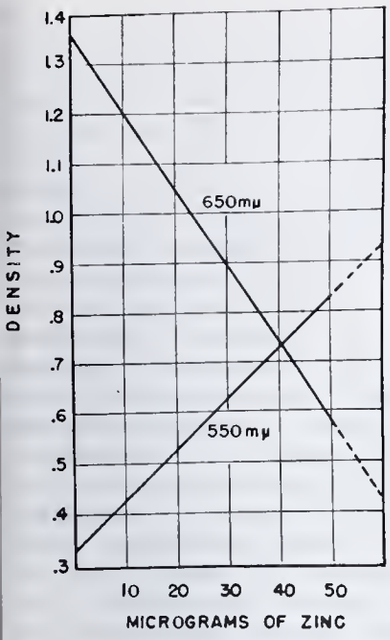

FIGURE 2. STANDARD CURVES FOR ESTIMATION OF ZINC
2.5-cm. cell

sized by Hubbard and used for the determination of mercury in urine (*13*). Since that time an improved method of synthesis has been developed whereby the reagent can be produced in greater purity and in a yield sufficient to facilitate more complete study of its properties. This method will be described in another paper (*14*).

The transmittancy curves for chloroform solutions of dithizone, of di-beta-naphthylthiocarbazone, and of the pure zinc-di-beta-naphthylthiocarbazone complex are shown in Figure 1. Absorption of the pure zinc complex is greatest at 550 mμ, in contrast to zinc dithizonate which has its maximal absorption at 535 mμ (*6*). Similar shifts of the maxima of absorption toward longer wave lengths were observed for the di-beta-naphthylthiocarbazone complexes of mercury, lead, and bismuth (*13*). A comparison of the transmittancy curves for chloroform solutions of di-beta-naphthylthiocarbazone and of its zinc complex shows that there are two regions suitable for photometric purposes—one at 550 mμ, the other at 650 mμ. An appreciably greater spread between the two curves is obtained at 650 mμ and therefore greater sensitivity of detection is possible when colorimetric measurements are made in this region. This increased sensitivity is further indicated in Figure 2, which shows standard zinc curves for the same concentration range obtained at the two wave lengths.

Effect of pH on the Efficiency of Zinc Extraction

The partitions between the aqueous and chloroform phases of the zinc complexes of dithizone and of di-beta-naphthylthiocarbazone, occurring at various pH values, are illustrated in Figure 3.

These data were obtained by extracting 50 micrograms of zinc from duplicate sets of salt buffer solutions at various pH values, with dithizone and di-beta-naphthylthiocarbazone. The salt buffer employed consisted of 30 ml. of zinc-free 20 per cent ammonium citrate solution and 5 ml. of a stock salt solution containing the ions found in solutions of ashed biological material. The salt solution chosen simulated a solution of ashed urine and each 5-ml. portion contained the ions present in 100 ml. of fresh urine (*3*). The pH was adjusted by adding either zinc-free ammonium hydroxide or hydrochloric acid, and the actual pH value obtained was determined by means of a glass electrode. The volume of each solution was brought up to 100 ml. with zinc-free distilled water before its extraction with the dithizone or di-beta-naphthylthiocarbazone. Extractions were carried out with single 10-ml. portions of the extraction reagents, which were of such strength as to contain 150 per cent in excess of the quantities theoretically necessary to combine with the zinc. Each solution was shaken for 1 minute and then was set aside for a short time to permit complete separation of the phases. The amount of zinc in each chloroform phase was then determined colorimetrically as described below.

Figure 3 shows that there is a decided difference in the extraction efficiencies of the two reagents. At a value of pH 8.3 both reagents extract zinc quantitatively. As the pH values increase above pH 8.5 there is a marked drop in the efficiency of the dithizone extraction, while that of di-beta-naphthylthiocarbazone remains unimpaired at least to pH 10.5. The literature contains a few data on the partition of zinc dithizonate between the aqueous and chloroform phases, but these are not in agreement with the authors' findings.

Walkley (*20*), employing a citrate–sulfuric acid–ammonium hydroxide solution, has reported unimpaired efficiency for a range of values from pH 8.0 to 9.5 with an excess of dithizone of only 37 per cent. In Hibbard's investigation (*10*) of the effect of pH on the partition of zinc, aqueous solutions were extracted (strength of extraction solution not given) with complete recovery of zinc throughout the range of pH from 7 to 10. Wichmann (*21*) has given a hypothetical curve which shows complete extraction from pH 6.5 to 10, but has made no claim as to its accuracy, since it had been drawn so as to be analogous to the lead extraction curve on the basis of fragmentary information gleaned from the literature. Biefeld and Patrick (*2*) have shown that other anions have a profound effect upon the lead partition in dithizone extraction and that this partition is also affected by the strength of the dithizone solution employed. Both of these factors account for the discrepancies between the authors' results and those of Walkley (*20*) and Hibbard (*10*). The latter investigators did not take into account all of the ions present in prepared biological specimens.

The authors' use of the salt solution buffer creates satisfactory conditions with respect to uniform efficiency of extraction for obtaining comparable and accurate results in the routine analysis of a wide variety of biological materials. The greater excess of dithizone employed in their method also tends to improve the extraction of zinc at higher pH values. These strong solutions are necessary to offset the effect of extraneous ions in the aqueous phase. The latter greatly retard the rate of extraction, so that a large excess of dithizone or di-beta-naphthylthiocarbazone must be employed to convert the zinc to the zinc dithizonate or the di-beta-naphthylthiocarbazone complex. As the pH value increases, more dithizone dissolves in the alkaline phase and this causes an increased solubility of zinc dithizonate in the same phase. The result is that the zinc and excess dithizone can be removed completely from alkaline media above pH 8.0 only by repeated treatment of the aqueous phase with fresh additions of dithizone followed by repeated washing with pure chloroform.

The insolubility of di-beta-naphthylthiocarbazone in alkaline aqueous solutions eliminates the necessity for critical control of the pH and reduces the manipulations required to extract the

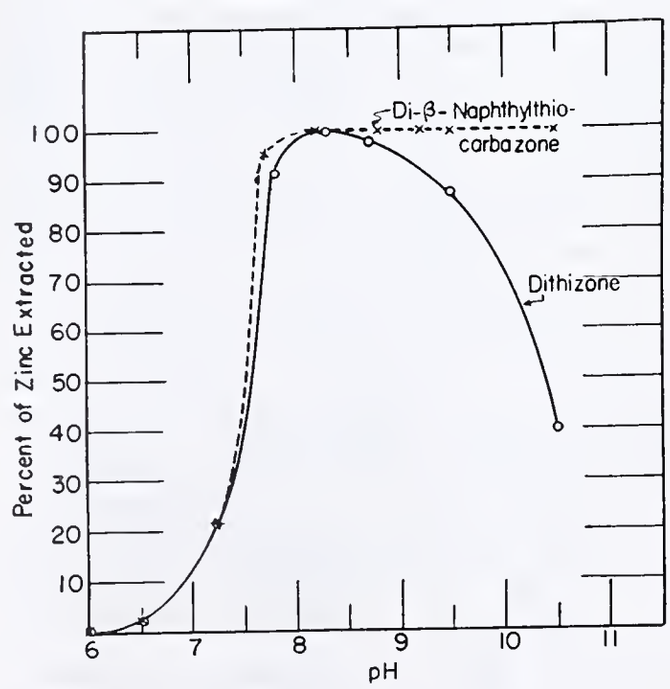

FIGURE 3. EFFECT OF pH ON ZINC PARTITIONS WHEN DITHIZONE AND DI-BETA-NAPHTHYLTHIOCARBAZONE EXTRACTIONS ARE MADE

zinc. The use of the reagent also limits the opportunities for loss of zinc to the mechanical factor of entrainment of droplets of the chloroform phase in the solution being tested. The small amount of the zinc–di-beta-naphthylthiocarbazone complex so held in the test solution may be reclaimed by a single treatment with pure chloroform or by the addition of a fresh portion of di-beta-naphthylthiocarbazone reagent following the addition of the last portion of di-beta-naphthylthiocarbazone which has retained its original color. The fact that di-beta-naphthylthiocarbazone is insoluble, however, makes it impossible to remove the excess of the reagent and therefore only "mixed color" colorimetric methods are applicable when di-beta-naphthylthiocarbazone is used as the extraction agent.

Elimination of Interferences

Chloroform solutions of di-beta-naphthylthiocarbazone extract the same metals as dithizone at pH 8 and therefore other metals beside zinc must be prevented from forming colored complexes. Sodium diethyldithiocarbamate added to the aqueous phase has been shown to be effective in preventing the formation of dithizonates of all metals but zinc (*6*, *12*). However, in the case of dithizone, carbamate prevents the complete extraction of zinc, the addition of as little as 12.5 mg. to the final estimation step resulting in the recovery of only 84 per cent of the zinc known to be present (*6*). This loss, added to that due to partition of zinc dithizonate in the final estimation step, results in standardization curves which do not obey Beer's law for the entire range of 0 to 30 micrograms of zinc (*6*). Therefore, when dithizone is used, reproducible results can be obtained only by strict control of the conditions of the procedure in handling the samples and standards, in order to maintain comparable losses of zinc in both.

The use of di-beta-naphthylthiocarbazone, on the other hand, results in a marked improvement in the extraction of zinc, the extraction being complete in the presence of even 50 mg. of carbamate. Standardization curves obtained with this reagent show no deviation from Beer's law for a range of values from 0 to 60 micrograms of zinc (Figure 2). This permits the addition of carbamate in the initial extraction step as well as in the final estimation step. The use of carbamate with di-beta-naphthylthiocarbazone in this fashion enables the analyst to fix the concentration range in the initial extraction step, a procedure which was not possible when dithizone was used.

Of the metals which react with carbamate, copper, nickel, cobalt, and bismuth (when present in large amounts) have chloroform-soluble colored complexes which affect the final estimation step unless they are removed. (They do not interfere markedly with the determination of the range of concentration in the initial extraction step.) Fortunately, the carbamate complexes of nickel, cobalt, and copper which may enter the chloroform phase are also insoluble in 0.02 *N* or 0.2 *N* hydrochloric acid, and therefore they remain in the chloroform phase when the latter is treated with the weak acid. The bismuth-carbamate complex is not quite so insoluble as the above metals, and a small quantity may enter the acid phase, particularly when large amounts of bismuth are present. When less than 100 micrograms of bismuth are present, however, a colored chloroform-soluble carbamate complex is not obtained. (Holland and Ritchie, *12*, also failed to obtain a colored carbamate complex when dealing with 20 micrograms of bismuth.) Since the final colorimetric estimation of zinc is carried out by adding sufficient ammonia to the acid phase and re-extracting with di-beta-naphthylthiocarbazone, interference by the small amount of bismuth which may have been carried along with the zinc is inhibited by the addition of more carbamate at this point.

Polarographic (Figure 4), spectrographic, and colorimetric studies of the acid wash of the initial di-beta-naphthylthiocarbazone extraction fail to show the presence of copper, nickel, cobalt, and mercury, but show that much of the lead and cadmium and a small amount of bismuth are carried along with the zinc. The lead-carbamate complex is colorless and therefore it does not interfere. The cadmium-carbamate complex is also colorless (*12*), but is evidently not so stable as the others and some of it (approximately 10 per cent) decomposes to yield cadmium ions. These combine with di-beta-naphthylthiocarbazone to give a colored complex which is measured along with the zinc complex. This action of cadmium has also been reported in connection with the extraction of zinc by dithizone-carbamate solutions (*6*). Since cadmium is rarely found in biological material, the interference will occur rarely, and in such instances, analysis must be carried out by polarographic means. Stannous tin and thallium (thallous) are prevented from interfering mainly because these metals are converted to the inactive higher valent forms in the procedures employed in the preparation of samples, but they also form colorless complexes with carbamate (*12*). Examination of spectra of the solution used in the final estimation step has failed to reveal the presence of tin (which may be present frequently and in large amounts in samples) or of thallium (which is rarely present). Silver, encountered occasionally, in the feces and in cooked foods, does not interfere. It is extracted by di-beta-naphthylthiocarbazone from alkaline solution, but its di-beta-naphthylthiocarbazone complex is stable in weak acid and therefore remains in the chloroform phase when the latter is shaken with 0.2 *N* hydrochloric acid. In addition, it forms a colorless complex with carbamate, so that any silver which may enter the final estimation solution in this form does not interfere.

In analyzing small volumes of solutions of prepared material of high ash content, some difficulty was encountered in extracting zinc unless the salt concentration was reduced by dilution with zinc-free water. For this reason it became a standard procedure to dilute the aliquot to 100 ml. with water before extraction.

It was also found that a single treatment of the initial di-beta-naphthylthiocarbazone extraction solution with 50 ml. of 0.02 *N* hydrochloric acid was insufficient to strip the zinc completely from the chloroform phase. Two factors were responsible for this—the presence of extraneous salts in the initial di-beta-

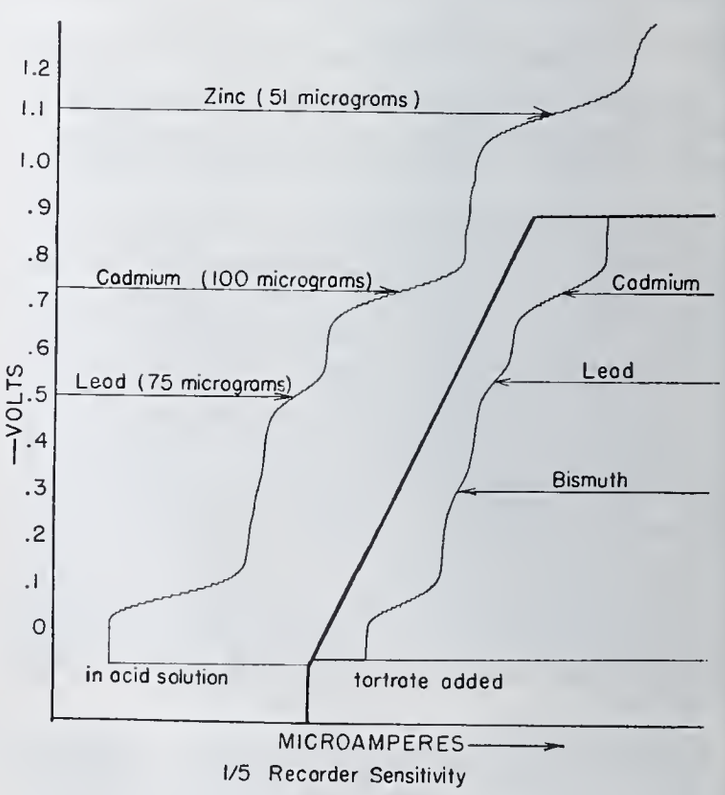

Figure 4. Polarograms Showing Effects of Certain Ions on Zinc Estimation

naphthylthiocarbazone extraction solution and the low strength of the acid employed. The first factor was eliminated by washing the initial chloroform phase with zinc-free water, and the second by employing 0.2 *N* hydrochloric acid in place of the 0.02 *N* acid. A single 50-ml. portion of the 0.2 *N* acid completely stripped 60 micrograms of zinc and yet failed to extract copper, cobalt, nickel, mercury, or silver. When the 0.02 *N* acid was employed it was not possible to strip more than 30 micrograms of zinc with a single 50-ml. portion of the acid.

When the zinc estimation is made polarographically, substances which reduce at potentials near that of zinc must be removed. This is true of nickel and cobalt, the waves of which may coalesce with the zinc wave if their concentration is high. The interference, therefore, is of a quantitative rather than a qualitative nature and any substance that will give a polarographic wave below, as well as near that of zinc, will also interfere if present in high concentration. Figure 4 shows a number of these possible interferences. Here it may also be seen that at high recorder sensitivities the amounts of these reducible substances must be diminished sufficiently to permit the zinc wave to appear on the chart. The initial di-beta-napthylthiocarbazone extraction as described below satisfactorily removes the zinc from iron, copper, nickel, cobalt, aluminum, phosphate, sulfate, and most of the bismuth and tends to reduce the quantities of lead and cadmium which are shaken into the acid phase employed for polarographic estimation. The extraction serves also to concentrate the zinc into small volume, thereby permitting greater accuracy in the analysis of materials low in zinc.

Procedures

Preparation of Samples. Samples are prepared for analysis by a combination of digestion and ashing procedures. The sample, consisting of weighed tissue or other material (10 to 20 grams) or 100 ml. of urine, is placed in a 200-ml. glazed silica evaporating dish. (When not in use the dishes are immersed in dilute nitric acid—1 part of nitric acid, sp. gr. 1.42, to 1 part of distilled water—in order to remove surface contamination.) Ten milliliters of redistilled nitric acid are added and the sample is taken to dryness on a hot plate. Organic matter is destroyed by igniting the sample in an electric muffle furnace maintained at 500° C. The complete destruction of organic matter may be hastened if the ash is treated with a little nitric acid from time to time, evaporated to dryness, and replaced in the muffle furnace. The clean ash is taken up in a little zinc-free hydrochloric acid and triple-distilled water. Insoluble residues are not filtered off but are carried along with the solution through the extraction steps. As little nitric acid should be employed as is consistent with rapid ashing, since it is not possible to free this reagent of zinc by distillation prior to its use. A blank analysis must be run with each series of samples, therefore, in order to correct for the zinc in the reagents.

Reagents. *Zinc-free distilled water* is obtained by redistilling double-distilled water in a Pyrex still.

Zinc-free ammonium hydroxide. Reagent ammonium hydroxide is distilled into triple-distilled water which is chilled in an ice bath.

Zinc-free hydrochloric acid is prepared by dropping a volume of concentrated sulfuric acid into an equal volume of concentrated hydrochloric acid in a Pyrex flask. A separatory funnel, its stem extending to a point just below the surface of the hydrochloric acid, is used for this purpose. The hydrogen chloride which is evolved is carried into the water by means of a delivery tube extending to a point just below the surface of the triple-distilled water kept in an ice bath. The strength of the zinc-free hydrochloric acid is obtained from standard tables, on the basis of the specific gravity as determined with a Westphal balance. From this acid, 0.2 *N* hydrochloric acid is made by dilution with triple-distilled water.

40 per cent ammonium citrate solution. Four hundred grams of citric acid are dissolved in water and sufficient ammonium hydroxide is added to make the solution just alkaline to thymol blue. The solution is made up to 1 liter with distilled water. Before use, the quantity required for 1 day is placed in a large separatory funnel, and is diluted with an equal volume of water. This 20 per cent solution is then shaken with a di-beta-naphthylthiocarbazone chloroform solution until the di-beta-naphthylthiocarbazone retains its original color.

Carbamate solution. One and a quarter grams of sodium diethyldithiocarbamate are dissolved in 100 ml. of triple-distilled water; 1 ml. = 12.5 mg. of carbamate. (This solution must be made up fresh daily.)

Extraction and standard di-beta-naphthylthiocarbazone solutions. DN solution 1 (0 to 5 microgram range). Twenty milligrams are dissolved in 1 liter of redistilled chloroform containing 10 ml. of absolute ethyl alcohol.

DN solution 2 (0 to 50 microgram range). Two hundred milligrams are dissolved in 1 liter of redistilled chloroform containing 10 ml. of absolute ethyl alcohol. (Both solutions are stored in brown bottles and are kept in the refrigerator when not in use.)

Chloroform Recovery. Used di-beta-naphthylthiocarbazone solutions are collected in glass-stoppered amber bottles and the chloroform is reclaimed in the manner described by Bambach and Burkey (*1*).

Apparatus. Pyrex ware is used throughout. Before use, extraction funnels should be rinsed with dilute nitric acid, followed by several rinsings with distilled water. Squibb-type separatory funnels are employed. These are of 150-ml. capacity with graduations at 5-, 10-, and all subsequent 10-ml. intervals up to 100 ml.

The densities of the colored solutions used in the zinc estimation were obtained with a photoelectric spectrophotometer, but any photometer, photoelectric or visual, which is equipped with a suitable filter and with 1-cm. and 2.5-cm. cells, may be employed. Filters with maximum transmission at either 550 or 650 mμ are preferred.

Polarographic estimations were made with the Leeds & Northrup Electro-Chemograph.

Initial Extraction Step. The entire sample or a suitable aliquot is placed in a 150-ml. separatory funnel. To this are added 30 ml. of 20 per cent ammonium citrate solution and 4 drops of 0.1 per cent aqueous thymol blue, followed by zinc-free ammonium hydroxide (sp. gr. 0.9) until pH 9.5 is reached. Four milliliters of carbamate solution (50 mg.) are now added and zinc-free water to the 100-ml. mark, and the solution is shaken for 1 minute with 5 ml. of extraction DN solution 1. [This will show whether the amount of zinc is less than 5 micrograms and permit the placing of the range. The color obtained is a faint violet (deeper shades of red indicate more than 5 micrograms) but some experience is necessary to recognize the range definitely.] Five milliliters of extraction DN solution 2 are added and the mixture is again shaken for 1 minute. If the range exceeds 5 micrograms, extractions with 5-ml. portions of the stronger solution are repeated until the last portion retains its original bluish-green color, each portion being drained into a second funnel before the next is added. (One should judge the color while shaking the solution, since the differences in color are more readily discernible in the shaken mixture than in the small volume of the chloroform phase.) The collected chloroform extracts are now washed with a 50-ml. portion of triple-distilled water and are drained into another funnel.

Di-beta-naphthylthiocarbazone entrained in the aqueous phase is removed by shaking the latter with one or two 5-ml. portions of pure chloroform, which are also added to the washed chloroform phase. The chloroform solution (or an aliquot containing not more than 50 micrograms of zinc) is now shaken with 50 ml. of 0.2 *N* hydrochloric acid and after the phases have been allowed to separate, the chloroform phase is discarded. Entrained di-beta-naphthylthiocarbazone is then removed from the hydrochloric acid by one or two washings with 5-ml. portions of pure chloroform. The 0.2 *N* hydrochloric acid contains all of the zinc freed of copper, nickel, cobalt, iron, mercury, silver, phosphates, aluminum, sulfates, and most of the bismuth and may be used for either polarographic or colorimetric estimation of the zinc.

Colorimetric Estimation Step. In another separatory funnel 45 ml. of ammonium hydroxide solution [50 ml. of zinc-free ammonium hydroxide (sp. gr. 0.90) diluted to 1000 ml. with zinc-free water and stored in the refrigerator when not in use] and 1 ml. of carbamate (12.5 mg.) are shaken with 5 ml. of extraction DN solution 1. (This treatment serves as a precautionary measure to remove zinc which may have been taken up from the glass by the weak ammonia during storage.) The DN extract is discarded and the ammonia solution is washed once with 5 ml. of pure chloroform, which is also discarded. Chloroform floating on the surface of the ammonia solution is allowed to evaporate or its removal is hastened by swirling the funnel and applying suction. The weak carbamate-ammonia mixture is then added to the funnel containing the 0.2 *N* hydrochloric acid extract of the zinc, from which floating chloroform has been removed as just described. The contents of the funnel are then mixed and the solution is shaken for 1 minute with 10 ml. of the proper extraction DN solution (DN solution 2 for the 0 to 50 microgram range and DN solution 1 for the 0 to 5 microgram range).

Table I. Recoveries of Zinc Added to Synthetic Urine Ash (3)

(Samples correspond to 100 ml. of urine)

Zinc Added	Zinc Found	Zinc Recovered by Colorimetric Method
Micrograms	*Micrograms*	*Micrograms*
0	2.2	....
0	2.2	....
1	3.0	0.8
1	3.2	1.0
5	7.0	4.8
5	7.0	4.8
30	33.0	30.8
30	32.0	29.8
50	52.0	49.8
50	52.0	49.8
500	500	498
500	490	488

Table II. Recoveries of Zinc

(In the presence of other metals which form DN complexes. Samples correspond to 100 ml. of urine)

Zinc Added, Micrograms	Other Metals Added, 100 Micrograms of Each	Zinc Recovered by Colorimetric Method, Micrograms
0	Hg, Pb, Cu, Bi	0
0	Hg, Pb, Cu, Bi	0
50	Hg, Pb, Cu, Bi	50
50	Hg, Pb, Cu, Bi	51 (polarograph 53)
50	Hg, Pb, Cu, Bi, Co, Cd	62 (polarograph 51)

For the estimation of 0 to 5 micrograms of zinc, 5 ml. of the DN extract are run quantitatively into a 25-ml. glass-stoppered cylinder and the volume is made up to 25 ml. with pure chloroform. A 2.5-cm. cell is then filled and the density of the solution is obtained at either 550 or 650 mμ.

When the 0 to 50 microgram range is used, 5 ml. of the DN extract are diluted to 100 ml. with chloroform and the density of the solution is obtained in a 1-cm. cell.

The amount of zinc present is then determined from standard curves (Figure 2) obtained by carrying known amounts of zinc in 0.2 *N* hydrochloric acid through the final colorimetric estimation step.

Polarographic Estimation. The 0.2 *N* hydrochloric acid extract obtained at the end of the initial extraction step is transferred to a beaker of suitable size and is then evaporated to a volume of 1 to 2 ml. After cooling, the acidity of the solution is adjusted until it is just acid to methyl red (zinc-free ammonia and 0.2 *N* hydrochloric acid) and the solution is transferred to a glass-stoppered 10-ml. Pyrex graduated cylinder, the volume being made up to 10 ml. with distilled water. A portion of this solution is placed in a polarograph cell, and after oxygen has been removed from the solution by bubbling tank nitrogen through it for 5 minutes, the polarogram is obtained from −0.8 to −1.3 volts. The zinc wave occurs at −1.1 volts (empirical, uncorrected) and its size is a measure of the concentration of the zinc in the solution. The quantity of zinc present in the 10-ml. volume is obtained by reading the value for the zinc step from a calibration curve obtained by treating known quantities of zinc in 0.2 *N* hydrochloric exactly as was done for the sample. Curves are derived for a number of recorder sensitivities at constant temperature and with a constant mercury drop rate. Recorder sensitivities of $^1/_5$, $^1/_{10}$, and $^1/_{40}$ will permit the determination of quantities of zinc varying from 5 micrograms to 1 or more milligrams per 10 ml. of solution.

Two other methods for evaluating polarograms may be used. The first method, employing an internal standard (4), has been found to be very accurate and eliminates the need for control of temperature, drop rate, or composition of the solution in the cell. In this method, 1 ml. of a cadmium solution (1 ml. = 0.06 mg.) is added to the beaker containing the residual of the 0.2 *N* acid evaporation. There is no change in the remainder of the procedure except that the polarograms are obtained from −0.5 to −1.3 volts. The ratio of the length of the zinc step to that of the cadmium (−0.72 volt empirical, uncorrected) is then read from calibration curves obtained by analyzing known amounts of zinc in the same manner. Obviously, the sample must be free from cadmium when this method is employed.

The other method, known as the standard addition method (11), is very convenient for use in the analysis of occasional samples, since it eliminates the need for the derivation and occasional checking of calibration curves. A polarogram is taken of a definite known volume of the test solution. After the addition of a known volume of a standard zinc solution, the polarogram is repeated and the concentration of the test solution is obtained from Hohn's formula (11):

$$\text{Concentration of unknown} = \frac{XvC_s}{x(V + v) + Xv}$$

where X = length of original step
x = increased length due to addition of the standard
C_s = concentration of standard solution
V = original volume of unknown in cell
v = volume of standard solution added

Reliability of the Methods

In Table I are recorded the recoveries obtained by the colorimetric procedure when known amounts of zinc were added to 5-ml. portions of a simulated urine salt stock solution (3) and the resultant samples were carried through the entire procedure, including the ashing step. The recoveries show no loss of zinc when samples are ignited at 500° C., nor does any loss result from the use of nitric acid as ashing agent. The blank of 2.2 micrograms shown in the table is due principally to the use of the nitric acid.

In Table II are listed recoveries for known amounts of zinc added to the stock urine salt solution (samples equivalent to 100 ml. of urine) which contained in addition 100 micrograms each of mercury, copper, lead, and bismuth. Also shown is a zinc recovery for a solution containing 100 micrograms each of mercury, bismuth, cadmium, copper, cobalt, and lead. The high result in this case is due to the presence of the cadmium. The polarograms in Figure 4 were obtained with a portion of the 0.2 *N* acid extract of this last preparation. These polarograms fail to show the presence of copper and cobalt and demonstrate that the lead, cadmium, and a small amount of bismuth are carried into the acid extract. The polarographic recovery of zinc in the presence of the above-mentioned metals was satisfactory, as may be seen from the value indicated in the figure and from Table II.

In Table III results obtained by the polarographic and colorimetric methods of analysis of typical materials are compared. The results obtained by the two methods are in satisfactory agreement.

Application of the Methods

These methods have been applied primarily to the analysis of biological material, but they can be applied to other materials as well. They have also been used to determine zinc in electrolytic

Table III. Comparison of Findings by Colorimetric and Polarographic Estimation

Material	Zinc Found: Colorimetric method	Zinc Found: Polarographic method
	Mg. per 24-hour sample	
Feces (1368)	10.5	10.5
Feces (1379)	11.8	11.3
	Mg. per liter	
Urine (680)	0.30	0.31
Urine (1523)	0.20	0.22
Urine (1529)	0.35	0.34
	Mg. per duplicate sample of food consumed in 24-hour period	
Food (1465)	6.4	6.4
Food (1503)	8.25	8.25
	Mg. per 100 grams of tissue (human)	
Cerebellum (790)	0.70	0.65
Lung (792)	0.65	0.70
Pancreas (794)	1.07	1.05
Kidney (798)	1.25	1.15
Blood (1958)	0.45	0.48
Blood (1972)	0.49	0.49
	Mg. per 10 cu. meter of air (in brass foundry)	
Air (10)	16.4	17.0
Air (11)	11.9	11.9

precipitator samples of foundry atmospheres and for the analysis of street dusts. They should be readily adaptable to the analysis of pharmaceuticals and soil samples.

Either method can be used conveniently over a wide range of concentrations, but the great sensitivity of the colorimetric method makes it the one of choice when the quantity of zinc present is known to be very small. Relatively large amounts can be handled more conveniently by polarographic means. The latter method is also more convenient when only occasional samples need be handled and is superior when cadmium is known to be present. Another point in favor of the polarographic method is its superiority whenever it may be desirable to determine lead, bismuth, and cadmium as well as zinc in the material under investigation. In such cases carbamate should not be added in the initial extraction step.

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Determination of Copper in Cast Iron by Direct Microelectrolysis

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This investigation of micromethods for the electrolytic determination of copper in nitric acid solutions of cast iron includes: the use of different kinds of apparatus and techniques; the effects of other metals; the effect of nitric, sulfuric, and perchloric acids; the effect of insoluble siliceous and graphitic residues; the molybdenum interference; and the error in sampling. A recommended procedure is given.

THE discovery of important technical properties in copper-iron alloys (*4*) has created a new interest in a rapid and accurate method of analysis for copper. Previously this was not a simple procedure but involved one or more preliminary chemical separations of the copper, followed by electrolysis or other method of determination (*5*, *6*, *9*). The chemical separation, in addition to being slow, was of questionable accuracy unless extreme care was applied.

Various reasons are given (*2*) for the necessity of separating other metals, particularly iron, from copper before it can be satisfactorily determined by electrolysis. (1) If much iron is present with the copper either the voltage used is not great enough to deposit all of the copper or, if it is, some iron usually deposits as well. (2) If nitric acid is present, the following phenomenon frequently occurs (*3*). By the time most of the copper has been deposited, the solution will have acquired a brown color and then the brown color will suddenly disappear, accompanied by a rapid solution of the copper. Further increasing the voltage will not redeposit the copper. This effect has been attributed to the oxidizing effect of ferric ions, nitric acid, ferric nitrate, and oxides of nitrogen. (3) Molybdenum, if present, deposits on the cathode as oxide.

All the variations described above have been observed when conducting, on a macro scale, the electrolysis of copper in nitric acid solutions containing much iron. Preliminary experiments showed that most of the difficulties disappeared when the determination was carried out with microapparatus and enough sample to give 0.3 to 2 mg. of copper.

This suggested the present investigation of micromethods for the electrolytic determination of copper in nitric acid solutions of cast iron. There have been studied: (*a*) the use of different kinds of apparatus and techniques; (*b*) the effects of other metals; (*c*) the effect of nitric, sulfuric, and perchloric acids; (*d*) the effect of insoluble siliceous and graphitic residues; (*e*) the molybdenum interference; and (*f*) the error in sampling.

Samples

A number of solutions containing various amounts of alloying metals were prepared in order to study the individual effect of each metal. After the method was developed it was tested with fifteen samples of cast iron or steel containing varying amounts of copper. These samples are described in Table I and are referred to below according to number.

Table I. Composition of Samples Analyzed

Sample No.	Type	% Cu	% Cr	% Ni	% C	% Si	% Mn	% Mo
1	Cast iron	8.41	4.62	20.20	2.56	1.96	1.22	...
2	Cast iron	8.04	(?)	...	...	...	...	...
3	Cast iron	7.38	2.85	17.32	2.36	1.82	1.08	...
4	Cast iron[a]	6.44	2.18	15.98	2.42	1.60	1.01	...
5	Cast iron	5.96	...	...	3.0	2.0	...	...
6	Cast iron	3.12	...	...	2.8	2.0	...	...
7	Cast iron	3.05	...	...	3.8	1.9	1.46	0.5
8	Cast iron	2.05	...	...	3.4	1.8	1.2	0.2
9	Cast iron	1.91	...	1.0	0.11	...	...	...
10	Cast iron	1.50	...	...	2.90	1.7	1.1	0.5
11	Cast iron[b]	1.44	0.018	0.13	2.86	1.84	0.61	...
12	Cast iron	1.09	1.0	...	...	...	1.0	...
13	Cast iron	0.38	...	...	...	...	...	...
14	Carbon steel[c]	0.267	0.26	0.25	1.03	0.39	0.345	...
15	Chrome-vanadium steel (0.22% V)	0.13	1.03	0.28	0.292	0.21	0.50	...

[a] Bureau of Standards sample 115.
[b] Bureau of Standards sample 5g.
[c] Bureau of Standards sample 35a.

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Table II. Electrolysis of Synthetic Samples

(5-ml. aliquots, H_2SO_4 + trace of HNO_3)

Solution	No. of Aliquots Run	Mean Weight of Cu Observed	Av. Deviation from Mean	Theoretical Weight of Cu	Error
		Mg.	*Mg.*	*Mg.*	*Mg.*
Cu	12	1.391	0.015	1.398	−0.007
Cu	4	1.492	0.015	1.502	−0.010
Cu-Fe	4	1.388	0.006	1.398	−0.010
Cu-Ni	7	1.410	0.007	1.398	+0.012
Cu-Cr	2	1.413	0.000	1.398	+0.015
Cu-Fe-Ni-Cr-Mn	6	1.382	0.017	1.398	−0.016

Table III. Electrolysis in Sulfuric Acid

(5-ml. aliquots, B. of S. sample 115, 6.44% Cu)

Sample	No. of Aliquots Run	Mean Weight of Cu	Av. Deviation from Mean	Theoretical Weight of Cu	Error
		Mg.	*Mg.*	*Mg.*	*Mg.*
B. of S. cast iron in H_2SO_4	8	1.292	0.005	1.291	+0.001
+ 1 ml. of HNO_3	1	1.291	...	1.291	0.000
+ 2 ml. of HNO_3	1	1.275	...	1.291	−0.016
+ 4 ml. of HNO_3	1	1.288	...	1.291	−0.003
+ 5 ml. of HNO_3	1	1.291	...	1.291	0.000

Apparatus

All the early work was done with the Pregl (*8*) apparatus. A separate study was first made by the authors (*7*) on the precision of the microelectrolytic determination of copper. In doing so the Pregl and Hermance-Clarke (*1*) electrolytic cells were compared. Results with the Pregl cell varied more than could be accounted for and were generally lower than with the Hermance-Clarke apparatus. In the latter cell the solution was drained through a stopcock with continuous addition of wash water, thereby maintaining the circuit until washing was complete. With this apparatus 1 to 2 mg. of copper could be deposited from 7 to 10 ml. of nitric or sulfuric acid solution with an accuracy of ±0.01 mg., a figure that has been used in the present work as the accuracy which may be expected when no interferences occur.

The Hermance-Clarke cell was slightly modified. The surrounding water jacket was omitted and the cell made slightly larger to accommodate 10 to 12 ml. of solution. Otherwise the electrodes, electrode support, and air stirrer were the same.

For smaller percentages of copper, samples 13, 14, and 15, it was necessary to use larger weights of samples. In order to avoid too high a concentration of other metals the solution of the sample was diluted to about 50 ml. and a correspondingly larger cell was used. It was constructed on the principle of the Clarke cell but with a funnel-shaped top to hold the extra solution. In such an apparatus all the solution circulates intimately between the electrodes.

The apparatus was equipped with voltmeter and ammeter with suitable resistances for changing the applied voltage.

The microbalance used was a Sartorius, air-damped type with protected beam. Its sensitivity was 1.35 micrograms per division.

General Procedure

No unusual precautions were taken. Air was bubbled through the solution at a rate of 2 to 3 bubbles per second. Cathodes were cleaned in hot dilute nitric acid, washed with water and alcohol, dried at 105° C. for 5 minutes, and allowed to stand 5 minutes before weighing. Bone-tipped forceps were used in handling the cathodes. Current and voltage were kept as nearly constant as possible during each electrolysis.

Effects of Other Metals

In preliminary work with alloys, results of wide variability and poor accuracy were obtained, but with synthetic solutions containing copper and other metals corresponding in concentration to the samples of cast iron described in Table I, the results (Table II) agreed well with the 0.01-mg. precision obtained with solutions containing only copper. The slightly low results whenever iron was present are not regarded as significant. This comparison was made in solutions containing sulfuric acid with only a trace of nitric, because it is well known that copper can be deposited from such a solution without interference. Deviation from theory could then be attributed to the effect of other metals.

Effects of Nitric, Sulfuric, and Perchloric Acids

It is usually recommended that copper be electrolyzed from a sulfuric acid solution. This is inconvenient because the sample must first be dissolved in an oxidizing acid which is then removed by evaporation. The effect of nitric acid was therefore determined by comparing (Table III) the results using sulfuric acid solutions with those containing various additions of nitric acid. The absence of any trend in the presence of nitric acid is apparent. Without any nitric acid, however, the evolution of hydrogen gas is excessive and produces a spotty deposit.

When nitric acid alone was used the results were equally good. In one set of experiments (Table IV) a copper solution was compared with one containing copper and iron, as a check on the frequent statement in the literature that low results are obtained with nitric acid solutions containing much iron.

In the second set of experiments in nitric acid the copper was determined in a cast iron. The results here (Table V) were slightly high (compare Table III, aliquots in sulfuric acid), which may in part be due to a slight amount of carbon and silica which was not filtered from the individual samples.

Finally, perchloric acid was tried as a solvent for the sample. The rate of solution of the sample was notably slower and evolution of hydrogen was excessive during electrolysis. Some blisters and cavities were observed microscopically on the deposit. When a small addition of nitric acid was made, gas evolution diminished greatly and the deposit was clear. The results obtained (Table VI) have as good precision as those from sulfuric or nitric acid.

Table IV. Electrolysis in Nitric Acid

(Synthetic samples, 5-ml. aliquots)

Solution	No. of Aliquots Run	Mean Weight of Cu Observed	Av. Deviation from Mean	Theoretical Weight of Cu	Error
		Mg.	*Mg.*	*Mg.*	*Mg.*
Cu	7	2.024	0.004	2.027	−0.003
Cu-Fe	7	1.997	0.005	2.003	−0.006

Table V. Electrolysis in Nitric Acid

(Hermance-Clarke apparatus. Individual samples Bureau of Standards cast iron 115, 6.44% Cu)

Sample Weight	Weight of Cu	Theoretical	Error
Mg.	*Mg.*	*Mg.*	*Mg.*
30.394	1.978	1.957	+0.021
30.946	1.993	1.993	0.000
35.947	2.355	2.315	+0.040
34.073	2.203	2.194	+0.009
29.617	1.934	1.906	+0.028
29.950	1.957	1.929	+0.028
28.499	1.856	1.835	+0.021
37.388	2.407	2.408	−0.001

Table VI. Electrolysis in Perchloric Acid

(5-ml. aliquots, B. of S. cast iron 115, 6.44% Cu)

Solvent	No. of Aliquots Run	Mean Weight of Cu	Av. Deviation from Mean	Theoretical Weight of Cu	Error
		Mg.	*Mg.*	*Mg.*	*Mg.*
$HClO_4$ + 3-minute boiling	3	1.246	0.003	1.292	−0.046
$HClO_4$ + 15-minute boiling	5	1.290	0.005	1.292	−0.002
$HClO_4$ + 15-minute boiling	2	1.282	0.003	1.292	−0.010

Effect of Insoluble Residues

It was apparent, through most of the work, that results with aliquot samples tended to be lower and in better agreement with the theoretical than with individual samples. In the latter case, any insoluble residue of silica and graphite was not filtered out but was added to the cell, in order to avoid filtration and consequent excessive volumes. When aliquot sampling was employed, the siliceous residue usually settled before samples were taken. It was suspected, however, that some suspended silica or graphite was being coprecipitated with the copper; therefore several experiments were made to check this point. Clear solutions were compared with those in which the suspension was purposely maintained at a maximum (Table VII). It is apparent that high results may be due to this effect. In separate experiments it was found that results were excessively high if the sample was ground unusually fine. In an effort to overcome this effect without the necessity for filtering or allowing the suspensions to settle, additions of gelatin and other organic reagents were made with the purpose of changing any charge on the solid matter and perhaps affecting its attraction by the cathode. Such attempts have been without success so far. The coprecipitation may therefore be largely a mechanical effect.

Interference by Molybdenum

Molybdenum when present was found to deposit on the cathode as the iridescent black sesquioxide, even at voltages as low as 2.2 volts. A solution of phosphoric acid or ammonium bifluoride prevented this. With bifluoride, however, a brownish precipitate formed on the cathode and additional nitric acid was necessary.

Table VII. Effect of Silica and Graphite

(Sample 1. Best chemical value, 8.41% Cu. Nitric acid solution, 5-ml. aliquots)

Solution	No. of Aliquots	Mean Weight of Cu Observed	Theoretical Weight of Cu	Error
		Mg.	*Mg.*	*Mg.*
1. Clear	6	1.713	1.684	+0.027
1. SiO_2, C suspended	1	1.749	1.684	+0.065
2. Clear (?)	4	1.773	1.726	+0.003
2. SiO_2, C suspended	3	1.837	1.726	+0.078
3. Clear	3	1.689	1.685	+0.004
4. Clear	2	1.714	1.707	+0.007

Table VIII. Determination of Copper in Molybdenum Cast Iron

Sample No.	Mo	Reagent	Theoretical Cu	Observed Cu
	%		%	%
10	0.5	NH_4HF_2	1.50	1.47
		NH_4HF_2		1.50
7	0.5	H_3PO_4	3.05	3.06
		NH_4HF_2		3.01
8	0.2	H_3PO_4	2.05	2.07
		NH_4HF_2		2.04

Table IX. Analysis of Sifted Samples

Sieve Size	Sample Weight	Weight of Cu Observed	Weight of Cu Theoretical	Error
	Mg.	*Mg.*	*Mg.*	*Mg.*
>20-mesh	21.03	1.774	1.769	+0.005
	20.77	1.748	1.747	+0.001
	22.09	1.845	1.858	−0.013
	19.92	1.680	1.676	+0.004
20–40	20.74	1.753	1.744	+0.009
	22.39	1.888	1.882	+0.006
	22.60	1.921	1.900	+0.021
	21.08	1.771	1.773	−0.002
<40	24.34	2.063	2.047	+0.016
	22.80	1.918	1.917	+0.001
	21.40	1.810	1.800	+0.010
	23.58	2.014	1.983	+0.031

Table X. Determination of Copper in Individual Samples of Steels and Cast Irons

Sample No.	No. of Samples	Approximate Sample Weight	Av. Deviation from Mean	Av. Cu Observed	Theoretical Cu
		Mg.	*Mg.*	%	%
1	12	20–24	0.008	8.44	8.41
2	4	20–23	0.007	7.98	8.04
4	6	20–26	0.016	6.45	6.44
5	4	21–23	0.002	6.07	5.96
9	4	40–43	0.012	1.98	1.91

Table XI. Determination of Copper in Aliquot Samples of Steels and Cast Irons

Sample No.	No. of Aliquots	Weight of Sample per Aliquot	Av. Deviation from Mean Weight of Cu	Av. Cu Observed	Theoretical Cu
		Mg.	*Mg.*	%	%
1	2	20	0.005	8.43	8.41
2	8	20	0.003	7.98	8.04
3	4	20	0.005	7.43	7.38
4	15	20	0.005	6.48	6.44
5	8	20	0.011	6.05	5.96
6	4	25–40	0.004	3.15	3.12
9	2	40	0.004	1.99	1.91
11	4	40–100	0.010	1.52	1.44
12	5	50–80	0.008	1.04	1.09
13	2	80	0.003	0.40	0.38
14	2	200	0.020	0.27	0.27
15	2	200	0.000	0.13	0.13

For a 5-ml. aliquot sample, 1.5 ml. of 85 per cent phosphoric acid or 0.2 gram of bifluoride plus 0.5 ml. of nitric acid was needed in order to prevent the deposition of molybdenum. A voltage of 2.5 volts was sufficient. Three samples of copper-molybdenum cast irons were analyzed with this modification (Table VIII).

Sampling Error

The sampling error is not important if samples as large as 20 to 40 mg. of 20-mesh or finer material are used. Samples were sifted and prepared in three sizes: coarser than 20-mesh, between 20- and 40-mesh, and finer than 40-mesh. Several determinations were made on each of the sifted samples. The results shown in Table IX indicate that individual sampling under these conditions is not a serious cause of error.

Recommended Procedure

Prepare a solution in the necessary solvent, using enough sample so that each 5 ml. will contain 0.3 to 2 mg. of copper. The sample should also contain 0.3 to 0.5 ml. of 70 per cent nitric acid. If the sample contains molybdenum add 0.15 ml. of 85 per cent phosphoric acid. Filter through a sintered-glass micro funnel into the electrolytic cell, rinse, and make up the volume to 9 to 10 ml. Add 3 drops of 95 per cent ethyl alcohol to eliminate spray, adjust the air stream used for stirring to 2 to 3 bubbles per second, and electrolyze at 2.5 to 2.8 volts for 20 minutes. Add 20 to 30 mg. of urea, rinse down the cell walls, and continue electrolysis for another 5 minutes. Slowly drain the cell, adding wash water simultaneously until the current has dropped to zero. Do not allow the voltage to exceed 3.5 volts during this operation. Withdraw the cathode, rinse with alcohol, dry for 5 minutes at 110° C., cool 5 minutes, and weigh.

Table X gives a summary of results obtained in this way using individual samples. Table XI shows similar results using aliquot samples.

Discussion and Summary

The successful electrolytic separation of copper from iron in the presence of nitric acid is attributed to the continuous removal of oxides of nitrogen by the stream of air used for stirring the solution. Nitric acid, which is the most commonly used solvent, therefore need not be removed before carrying out the electroly-

sis. Some nitric acid is, of course, desirable since the nitrate ions prevent excessive evolution of hydrogen gas which would cause a blistered deposit. Sulfuric and perchloric acids do not interfere with the deposition.

The deposits obtained according to the recommended procedure are firm and free from blisters. Qualitative tests have shown them to be free from iron.

The principal interfering metal commonly present is molybdenum, which deposits with the copper as the iridescent black oxide. This is satisfactorily avoided by addition of phosphoric acid or of ammonium bifluoride and some extra nitric acid. Tungsten is ordinarily considered an interfering element, but since it is removed as the insoluble oxide after the sample has been treated with nitric acid, it does not affect the electrolysis. Carbon and silica have been found embedded in the deposit and the deposits were notably heavy in the cases where these insoluble materials were left suspended in the solution of the sample. Simple filtration through a fritted glass microfilter, as part of the process of transferring the sample to the electrolytic cell, has eliminated this difficulty.

The precision of the separation of 0.3 to 2 mg. of copper from aliquot samples of solution of copper containing steels and cast irons is of the order ±0.01 mg. This value is only slightly larger when individual samples are analyzed, showing that sampling is not a serious problem.

The time required for depositing 0.3 to 2 mg. of copper under the conditions described in this paper is not in proportion to the time required for macro amounts of copper, but is relatively greater. Although it has not been possible to shorten the time to less than 25 to 30 minutes, the procedure is definitely more rapid than those employing chemical separations.

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Microdetermination of Glycolic and Oxalic Acids

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A quantitative colorimetric micromethod for glycolic acid has been devised from a sensitive qualitative spot test based on the color produced with 2,7-dihydroxynaphthalene in acid solution. The same reaction may be employed for the quantitative microdetermination of oxalic and glyoxylic acids after reduction with powdered magnesium. Glycolic acid appears to be an intermediate product in the oxidation of ascorbic to oxalic acid.

FOR use in a study of the stabilizing action of ascorbic acid in certain fats (*2*), a micromethod for the quantitive estimation of oxalic acid was sought. Oxalic acid is a postulated oxidation product of ascorbic acid (*1*) and might be the substance ultimately responsible for its antioxygenic action. On controlled reduction with powdered magnesium, oxalic acid yields glycolic acid (*3*).

This paper describes the application of a color test for glycolic acid to the microestimation of glycolic and oxalic acids. By a suitable modification glyoxylic acid can also be determined, since it is likewise reduced to glycolic acid with powdered magnesium (*3*). When glyoxylic acid is present with oxalic acid in the same solution, the latter can be removed by precipitation from neutral solution by gypsum water.

When a solution of glycolic acid reacts with a solution of 2,7-dihydroxynaphthalene in concentrated sulfuric acid, a violet-red color is produced on heating; according to Eegriwe (*3*), who first described this reaction, the color probably depends on the condensation of formaldehyde, split off from the glycolic acid, with dihydroxynaphthalene to form tetrahydroxydinaphthylmethane; this colorless product is then gradually oxidized to a deep red dyestuff. None of the more common organic acids interfered with the test, including formic, acetic, oxalic, succinic, tartaric, citric, benzoic, and salicylic.

Color measurement is made on a calibration curve prepared from known solutions of glycolic acid and read in a Coleman spectrophotometer at 530 mμ or a Klett-Summerson photoelectric colorimeter with filter No. 52. Figure 1 shows the spectral transmittance curve of several concentrations of glycolic

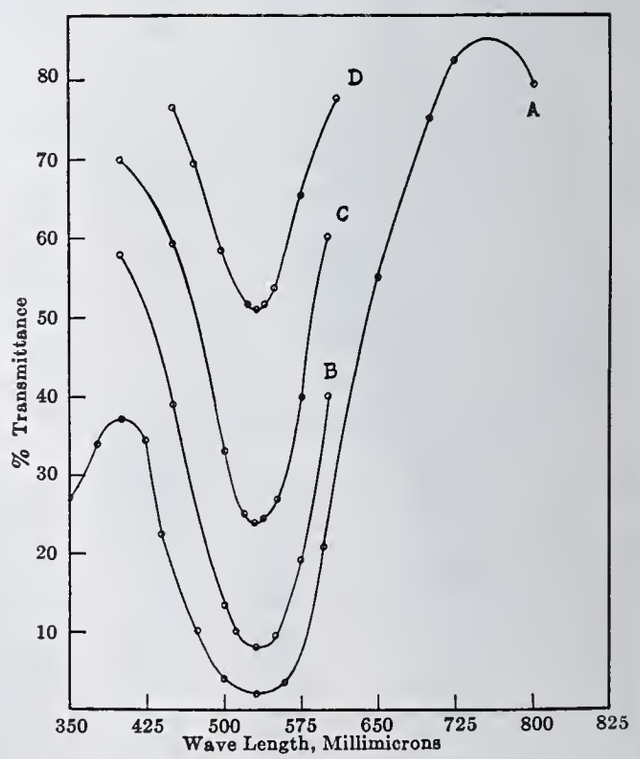

FIGURE 1. SPECTRAL TRANSMISSION CURVE

Micrograms of glycolic acid: *A*, 40; *B*, 20; *C*, 10; *D*, 5

acid. The calibration curves of glycolic acid (logarithmic) and of oxalic acid were both straight lines within the ranges used.

Determination of Glycolic Acid

The solution to be analyzed should be 2 *N* with sulfuric acid and should contain not over 100 micrograms of glycolic acid per ml. Into a 15-ml. conical glass-stoppered centrifuge tube, 0.2 ml. of this solution is introduced with a serological pipet, and 2 ml. of 0.01 per cent solution of 2,7-dihydroxynaphthalene in concentrated sulfuric acid are added from a microburet, the tube being kept in a cold-water bath during the addition of the reagent. The mixture is made homogeneous by rotating the tube between the hands, and the tube is then placed in a boiling water bath for 20 minutes, removed, and allowed to cool to room temperature. After cooling, the solution is diluted with 4 ml. of 2 *N* sulfuric acid and shaken, lightly at first, until the heat of reaction has subsided. The solution is made homogeneous by vigorous shaking and the color is then read.

The method is very flexible; after the color has been formed, practically any dilution volume can be used if it is preferable to reduce the sensitivity of the method so that higher concentrations of glycolic acid can be determined. Even the volume of test solution can be changed within narrow limits.

Determination of Oxalic Acid

This differs from the preceding only in that to the original sample as measured out (0.2 ml. of a 2 *N* sulfuric acid solution containing not more than 200 micrograms of oxalic acid) 5 mg. of powdered magnesium are added; after rotating between the palms of the hands, the tube is allowed to stand for an hour. Then with the tube in cold water, 2 ml. of the dihydroxynaphthalene solution are added, and after being made homogeneous, the solution is heated on the boiling water bath for 20 minutes, removed, and allowed to cool to room temperature. It is then diluted with 4 ml. of 2 *N* sulfuric acid, mixed, and the color is measured.

The calibration curve shows that Beer's law is obeyed from 0 to 200 micrograms of oxalic acid, and even with this range the method is sensitive at low concentrations (0 to 20 micrograms).

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Micromethod for Estimation of Sulfonamides

Semipermanent Color Standards

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As a supplement to the method for sulfonamide determination previously reported (*1*), a simple procedure has been worked out, using color standards prepared from easily available, water-soluble dyes. It is felt that this modification will be of use especially in areas remote from laboratory facilities. Acceptable accuracy has been obtained to blood levels of 15 mg. per cent (for sulfathiazole). By averaging the results of several observers, it was found that the error (for sulfathiazole) was ±0.5 mg. per cent to levels of about 6 mg. per cent, and ±1 mg. per cent at higher levels to 15 mg. per cent.

The advantages of the parent micromethod have been retained, though the procedure has been simplified. The number of manipulations and the required apparatus are minimal.

The volume of blood needed (0.3 ml.) may be taken from the finger tip, if the hand of the patient has been vigorously washed to remove traces of the sulfa drug. Flat-bottomed 10-ml. vials (18 × 46 mm.), calibrated for the purpose of the method, are to be preferred to centrifuge or test tubes.

The standards of known sulfonamide concentration may be made from any of the sulfonamides, and the values for the others obtained by using molecular weight corrections. The values obtained for sulfathiazole may be multiplied by 0.68 to convert to values for sulfanilamide, while those for sulfapyridine are the same as for sulfathiazole.

Procedure. The same reagents and procedure are used as in the parent method (*1*), but only the blood and precipitating solution volumes need be measured with accuracy. The nitrite and dye solution (2 drops or 0.1 ml.) may be added from a dropper. Comparisons of the tubes from the determinations with the standards are made visually, always using vials of uniform shape. It was noted that the eye best measures the intensities of transmitted light if the absorbing substances be held 1 to 3 cm. from the eye. One should look through the solution.

Color Standards for Comparisons. Semipermanent fuchsin–methyl violet color standards were prepared as follows: Acid fuchsin (0.100 gram) was dissolved in 250 ml. of distilled water, and this was diluted 1 to 8 with water, giving a solution containing 0.00005 gram in 1 ml. Methyl violet (0.100 gram) was dissolved in 250 ml. of water, and 2 ml. of this solution were made up to 250 with water (0.0000032 gram in 1 ml.). These solutions were mixed and diluted as shown in Table I to give color intensities corresponding to the indicated amounts of sulfathiazole.

Table I. Preparation of Color Standards

Standard	Fuchsin Solution	Methyl Violet Solution	Water
Mg. %	*Ml.*	*Ml.*	*Ml.*
2	0.9	9.1	36.0
3	0.9	9.1	25.3
4	0.9	9.1	19.0
5	0.9	9.1	14.7
6	0.9	9.1	11.2
7	0.9	9.1	8.8
8	0.9	9.1	7.0
9	0.9	9.1	5.4
10	0.9	9.1	3.9
11	0.9	9.1	2.9
12	0.9	9.1	2.1

Several combinations of red and violet water-soluble dyes gave shades almost identical to that of the azo color formed in the reaction. Colors were matched with the eye. The dye combination is, of course, bleached by sunlight, and those in use lasted for about a month. Standards should be kept in the dark when not in use. Stronger solutions of the dyes keep longer than the weak ones, and may be kept for subsequent diluting.

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Microdetermination of the Formyl Group

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WHILE the microdetermination of the acetyl group is now widely used, little attention has been paid to analysis of the formyl radical. Usually identification of the formyl derivative by means of carbon and hydrogen analysis is sufficient, but in certain critical cases an analysis of another constituent may be desirable.

The acetyl determination should be valid for all volatile acyl groups, but since no reference to the application of this method to the formyl group was found in the literature, this determination was studied.

Because it was felt that the reducing properties of formic acid might serve to distinguish it from the other volatile acids, the question of whether formic acid and bromine react quantitatively in 0.01 *N* solutions was investigated. It was found that the formyl group can be determined under the same conditions as the acetyl group. The determination can be made specific by treating the distilled formic acid with an excess of bromine.

Formic acid solution, 0.01 *N*, was prepared and standardized by means of excess potassium iodate and potassium iodide, and 0.01 *N* bromine solution was prepared and standardized by titration of iodine from potassium iodide. This solution must be stored in a refrigerator and its factor checked frequently. An excess of the bromine solution was added to a measured amount of formic acid in a stoppered flask and shaken thoroughly, and after 5 minutes the excess bromine was determined (Table I).

TABLE I. DETERMINATION OF FORMIC ACID

0.0102 *N* Formic Acid	0.00982 *N* Bromine Solution	Calculated Excess	Observed Excess	Difference
Ml.	*Ml.*	*Ml.*	*Ml.*	*Ml.*
10.00	15.00	4.61	4.58	+0.03
10.00	15.00	4.61	4.64	−0.03
10.00	15.00	4.61	4.57	−0.04
5.00	10.00	4.81	4.82	+0.01
5.00	10.00	4.81	4.78	−0.03
5.00	10.00	4.81	4.77	−0.04

TABLE II. DETERMINATION OF FORMYL

Substance	Formyl Found as Formic Acid	Formyl Found as Reducing Agent	Formyl Calculated
	%	%	%
Triformyl cholic acid	17.19 17.30	17.33	17.68
Diformyl desoxycholic acid	12.56 12.66	12.43	12.94
Cholesteryl formate	7.01 6.88	7.25	7.00
S-Benzyl-*N*-formyl-*dl*-cysteine	12.38	...	12.15
S-Benzyl-*N*-formyl-*d*-cysteine	12.20 12.05	...	12.15
S-Benzyl-*N*-formyl-*l*-cysteine	12.15 12.20	...	12.15
Methyl formaniline	21.36 21.32	...	21.48

Determinations were run according to the Elek-Harte method for acetyl (*2*) on several formyl derivatives whose purity was checked by melting point and carbon and hydrogen analysis (Table II). In a few instances where there was available material, the modified method using the liberated formic acid as a reducing agent was followed. It was observed and substantiated by the senior author (*1*) that correction for the amount of sulfur dioxide liberated from the saponifying agent is seldom necessary, if the procedure is carefully controlled. Therefore, freshly distilled water can be used in the receiver where there is a question of the nature of the acid group. In this case, the distillate is quantitatively transferred to a stoppered flask, the properly prepared bromine solution is quickly added in excess, and the excess is titrated as described. If both formyl and acetyl may be present, a total acid titration should first be performed, preferably on a separate sample or on an aliquot of the distillate.

Acknowledgments

The author wishes to express his thanks to Oskar Wintersteiner for his advice and interest in this work.

The cysteine derivatives were kindly furnished by J. A. Stekol.

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Pipetting Apparatus

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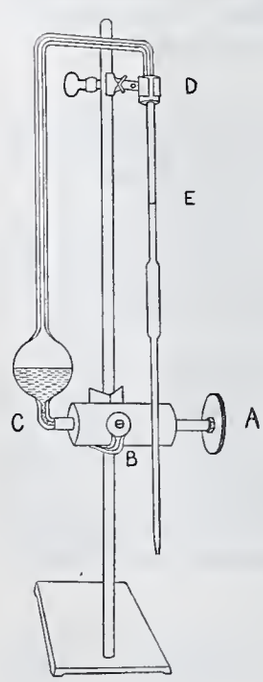
DURING a recent research, it was necessary to withdraw accurately aliquots of 2 and 3 cc., respectively, from a two-phase liquid system containing 3 cc. of aqueous solution and 4 cc. of a chloroform solution. The solute, whose concentration in each of the two phases was to be determined, was highly toxic. In addition to the hazards involved in carrying out the operation by the usual pipetting technique, it was difficult to obtain a clean separation of the layers. The apparatus described below, while originally designed to fill these needs, has, because of its simplicity and ease of operation, been found extremely useful for all pipetting operations, and particularly for pipetting strong acids and bases, toxic liquids, and volatile solvents. The apparatus provides smooth, accurate control, and the speed of pipetting is comparable to that by the usual technique.

The brass cylinder, *B* (2.5 cm., 1 inch, in inside diameter), receives a threaded brass plug, *A* (threaded surface 2.5 cm., 1 inch, long); 16 threads per inch have been found convenient. The capacity of the cylinder assembly is 25 cc. Pyrex capillary tubing (2-mm.), joined to the opposite end of the cylinder by means of a suitable cement (Shawinagan resin, Picein, or DeKhotinsky cement), is sealed to a glass bulb, *C*, of 25-cc. capacity, and the latter joined to a further length of capillary tubing as shown in the diagram. *D* is a No. 1 one-holed stopper which receives the pipet. About 30 cc. of glycerol are introduced into the apparatus; air trapped in *B* can be removed by holding the apparatus on its right side and turning up plunger *A*. The glycerol besides lubricating the threaded surface acts as a convenient air block, making the apparatus airtight.



To operate the apparatus, the pipet to be used is moistened and inserted into stopper *D*. By turning *A* counterclockwise, the liquid to be pipetted is lifted slightly above the pipet mark, *E*. With the tip of the pipet resting against the side of the vessel containing the liquid, *A* is turned slowly clockwise until the liquid level reaches mark *E*. Delivery is then effected by clockwise rotation of *A*. Any volume of liquid from 25 cc. to less than 0.01 cc. can be accurately measured.

A small-diameter, knurled extension attached to disk *A* may be found convenient for major adjustments, and will considerably reduce the time required for operation.

NOTES ON ANALYTICAL PROCEDURES

Substitute for Benzene in the Determination of Acid Numbers

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The aromatic petroleum solvent Solvesso No. 1 is submitted as a substitute for benzene in acid number determinations on oils, resins, and related products. The physical properties of the two solvents are tabulated and a comparison is presented of their action as solvents in the acid number titration of several paint vehicle ingredients.

IN THE determination of the acid number of oils, resins, and varnishes as outlined in the A. S. T. M. standards on paint, varnish, lacquer, and related products, the customary solvent is a mixture of C. P. benzene and 95 per cent ethyl alcohol (*1*). Essentially, the procedure involves the titration of free acid with standardized alcoholic potassium hydroxide using phenolphthalein as indicator, and the results are reported as milligrams of potassium hydroxide per gram of oil, resin, or varnish.

The authors' laboratory has frequent occasion to run acid number tests, and had standardized on a mixture of equal parts of C. P. benzene and denatured anhydrous ethyl alcohol. Owing to the exigencies of war, it was found advisable to secure a substitute for the benzene.

A study of the literature disclosed that a number of other solvents and solvent combinations had been investigated. Methyl, isopropyl, and amyl alcohols were substituted for ethyl alcohol (*2*, *4*, *6*); however, these substitutions did not eliminate the necessity of using benzene to ensure a suitable range of solubility. Ether and chloroform (*3*, *4*) were suggested in combination with ethyl alcohol, but the investigation covered a narrow range of materials and did not indicate their usefulness in the case of synthetic resins and natural gums.

Because of its availability and physical properties, the solvent selected for examination was the aromatic petroleum product, Solvesso No. 1, supplied by the Colonial Beacon Oil Co. (*5*). A comparison of its physical and solvency characteristics with those of C. P. benzene is presented in Table I. Common paint vehicle ingredients were checked for acid number in the following solvent mixtures (results are given in Table II):

A. 50% C. P. benzene
   50% denatured anhydrous alcohol
B. 50% Solvesso No. 1
   50% denatured anhydrous alcohol
C. 66.6% Solvesso No. 1
   33.3% denatured anhydrous alcohol

The mixture of 2 parts of Solvesso No. 1 to 1 part of denatured anhydrous ethyl alcohol proved to be an effective solvent for the materials commonly checked for acid number. In the case of heavy bodied linseed oil, the standard benzene-alcohol mixture turned extremely cloudy during titration, while mixture C maintained a clear solution throughout the titration, permitting a more rapid introduction of potassium hydroxide solution and yielding a sharper end point. In all determinations, no interference could be detected with either the titration or the end point, and it is concluded that the Solvesso No. 1–alcohol combination is a satisfactory substitute for the benzene-alcohol standard.

TABLE I. PHYSICAL PROPERTIES OF C. P. BENZENE AND SOLVESSO NO. 1

	C. P. Benzene	Solvesso No. 1
Specific gravity	0.884	0.815
Boiling range	80–80.5° C.	93.3–135° C. 90% off at 118.8° C.
Kauri-butanol value	120	72.5
Mixed aniline point	7.3° C.	26.7° C.

TABLE II. ACID NUMBER DETERMINATION USING SOLVENT MIXTURES

Sample	Solvent Mixture A	B	C
Oils			
Raw linseed oil	3.8	3.8	3.8
Bodied linseed oil, high acid	19.1[a]	19.2[a]	19.2
Resins			
Gum rosin	168	170	169
Limed wood rosin	81.0	82.3	81.1
Maleic resin	14.5	14.5	14.5
Alkyd resin	14.7	14.6	14.7
Ester gum	7.9	8.0	8.0
Run kauri	108	107	107
Run East India	16.5	16.7	16.7
Manila	123[a]	123[a]	124[a]
Coumarone-indene resin	Neutral	Neutral	Neutral
Varnishes			
Limed wood rosin			
Linseed oil, 50 gallon oil length	17.0[a]	17.0[a]	16.9
Ester gum–linseed oil, 20 gallon oil length	2.9	2.8	2.8
Phenol formaldehyde–tung oil–linseed oil, 25 gallon oil length	9.7	9.6	9.7

[a] These samples yielded cloudy solutions.

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Sintered Pyrex and Soft Glass Sections in Tubes and Crucibles

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SINCE the report on porcelain filter mats (*10*) and Pyrex filter mats (*11*), many contributions have appeared on the construction and use of Pyrex sintered ware. In general the procedures suggested have involved sealing in place a prepared and shaped disk of sintered Pyrex, porcelain, or Alundum under controlled heating in a furnace (*1, 2, 3, 5, 7, 8*). In others the sintered-glass sections are prepared in tubes in open flames (*4, 6, 9*).

The reason for so many contributions is no doubt the efficiency and ease of use of sintered-glass or porcelain ware in gas dispersers, gas wash bottles, mercury filters and valves, stills, absorbers, extraction thimbles, filters for fine precipitates, and aerators.

The advantages of construction of a sintered mat directly in the tube or crucible in one operation have been demonstrated by the senior author. The graded glass may be packed in any part of the tube and sintered in place. The authors have not observed any reference to their method and therefore add this report on procedure and technique for the benefit of those who wish to construct their own apparatus for preliminary or exploratory observations.

Preparation

The glass was ground in a steel mortar and graded by means of a set of sieves, 10- to 200-mesh. The graded glass was treated with 6 *N* hydrochloric acid, washed, and dried. For the first heating the mat was prepared by placing an asbestos disk at the lower level and holding it in place by fine asbestos fiber packed from below. The graded glass was poured on to the desired thickness. For the construction of a filter mat this first layer consisted of a mixture of 40 per cent of 40-mesh, 35 per cent of 80-mesh, and 25 per cent of 120-mesh. The mat was wetted with a few drops of *N* sodium hydroxide (sodium silicate solution, specific gravity 1.036, also served as a suitable flux). Without the flux many mats failed to fuse uniformly to the wall of the tube or crucible. After this sturdy mat had been sintered in place, the process of controlling porosity followed. The finer grades (130- to 200-mesh) were kept suspended in the diluted fluxing solution and pulled into the interstices by suction, thus building up an effective filter bed. The smaller particles were then sintered.

Control of Sintering Process

The approximate temperature or cone number required for sintering was determined with cones made from the glass by drawing a short section of tubing to a point. These glass "cones" were placed in the furnace along with a selected series of pyrometric cones, or a thermocouple. The approximate temperature at which the glass would sinter was thus determined in terms of cone number or thermocouple range. For the work reported here cone No. 0.020 was found suitable for the soft glass and cone No. 0.015 for the Pyrex, while 625° C. (thermocouple) was suitable for the soft glass and 745° C. for Pyrex.

The glassware was made ready for the furnace by packing the glass tubes or crucibles in fire-clay crucibles with fine asbestos fiber. Distortion was reduced to a minimum by maintaining the tubes in a vertical position by loose uniform packing. Sturdy sintered mats were obtained with soft glass when the temperature was allowed to rise slowly to 625° C. and held there for 15 minutes and with Pyrex when the temperature was held at 745° C. for 25 minutes.

Cleaning of Sintered Mats

The sintered mats were washed with water, dried, and weighed (weight A, Table I). Any soluble substances remaining from the sodium hydroxide or sodium silicate treatment were extracted with 6 *N* hydrochloric acid in the following manner: The mat was placed in the acid for 24 hours, then washed, dried, and weighed (weight B, Table I); the process was repeated to determine further loss (weight C).

Pressing the mats with a stirring rod indicated no apparent loss in sturdiness from the acid extraction. Walls of split mats examined under a low-power microscope, equipped with an illuminator, showed that the porosity was uniform and that the structure was continuous.

The method of preparing built-in sintered mats has been successful for a wide variety of grades of powdered glass. Sections limited by the size of muffle of the furnace can be joined to larger pieces of glass. The coarser grades can be heated to slightly higher temperature with resulting increase in sturdiness. The authors suggest this method only as a device for preparing sintered-glass mats during exploratory experimentation in regular laboratory procedure or research. The amateur glass worker should rely on experts for the construction of permanent glass equipment of special design.

TABLE I. WEIGHT LOSSES FROM HYDROCHLORIC ACID TREATMENT

Thickness of Mat *Cm.*	Weight A *Grams*	Weight B *Grams*	Loss *Mg.*	Weight C *Grams*	Loss *Mg.*
Soft glass					
1.0	8.5895	8.5852	4.3	8.5852	0.0
0.9	6.9514	6.9482	3.2	6.9483	−0.1
1.0	8.3727	8.3692	3.5	8.3692	0.0
1.1	7.6223	7.6193	3.0	7.6195	−0.2
Pyrex					
0.7	6.3704	6.3690	1.4	6.3690	0.0
0.5	5.8145	5.8131	1.4	5.8130	0.1
0.7	4.8661	4.8647	1.4	4.8648	−0.1
1.0	8.1299	8.1278	2.1	8.1277	0.1

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The Use of Filter Paper Disks with Gooch and Porous-Type Crucibles in Routine Filtrations

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THE acute shortage of Gooch-grade asbestos fiber has made it necessary for all laboratories to devise means for conserving their diminishing supply, or to turn to other less satisfactory means of quantitative filtration. The following procedure has proved of value as a time-saver and as a means of conserving asbestos fiber, especially when routine filtrations of precipitates such as barium sulfate or magnesium pyrophosphate are in order.

Where a limited supply of asbestos is still available, a Gooch crucible is made up in the ordinary manner, ignited, cooled, and weighed. Just before filtering, a filter paper disk (24 mm. in diameter for 30-ml. crucibles) is placed on the mat in the crucible and washed once with distilled water to secure it as firmly as possible against the mat. [Carl Schleicher & Schüell Co.'s No. 895-E ashless filter mats have proved very satisfactory for the use described.] The filtration is then carried out in the usual manner, and the crucible is ignited, cooled, and weighed. Immediately after weighing, the precipitate is removed mechanically, and the crucible is reweighed and thus is immediately ready for reuse.

To remove the precipitate, the crucible is inverted and gently tapped. Most of the nonadhering precipitate will drop out, since the filter paper disk prevents the adherence of the precipitate to the filter mat, whether it be asbestos, fritted glass, or porcelain. Should any precipitate adhere, after it is gently loosened with the aid of a spatula, it can be brushed out with a camel's-hair balance brush.

The procedure with modifications has been applied with equal satisfaction in the use of the porous-type filtering crucible and the time saved in cleaning has been considerable. In using this type of crucible the filter paper disk (24 mm. in diameter for 30-ml. crucibles) is inserted just before filtering and, as with the Gooch crucible, the precipitate is removed mechanically just after weighing. The crucible is then reweighed and ready for reuse.

The success of the use of the filter paper disks seems to be brought about by the following action during ignition within the crucible. The paper prevents adherence of the ignited precipitate to the asbestos mat or to the filtering medium of the porous-type crucible by the expulsion of the volatile organic matter during the combustion. It also prevents the clogging of the asbestos mat or the filtering medium by the material being filtered.

The procedure can be used for any ignited precipitate, whether it be capable of removal by solution or not. The removal of most filtered solids by solution is time-consuming, and doubtful as to completeness. The method described provides a crucible, weighed and ready for another filtration in very short order, with no impairment to the filter mat by occlusions.

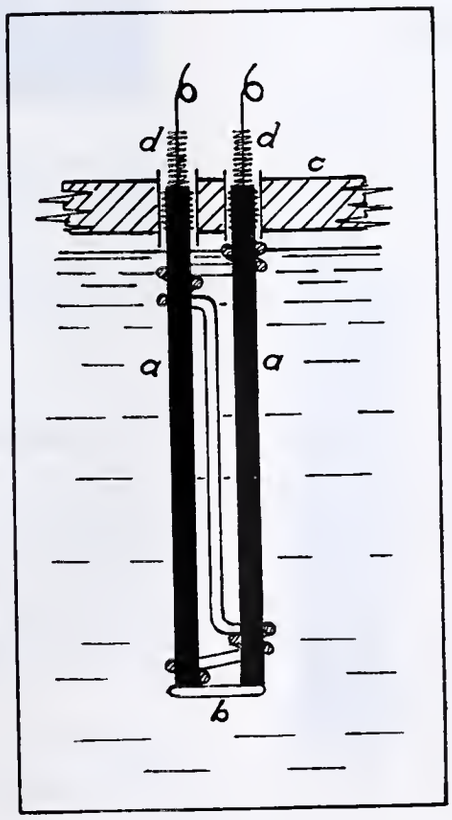
Electric Heating Unit

GABOR B. LEVY, Schenley Research Institute, Lawrenceburg, Ind.

IT IS frequently necessary to provide apparatus with a bath containing boiling water in order to maintain a temperature of approximately 100° C. In order to heat up and maintain the liquid at the boiling point, an electric heater can be used to advantage.

Since it is rather difficult, at present, to procure suitable heaters, the construction of a heating unit in the laboratory may often become imperative. A simple electrical heater can be assembled from readily available materials by using the liquid of the bath as resistance. In such heaters, the rate of boiling is controlled by the quantity of electrolyte present, the dimensions and distance of the electrodes, and the voltage applied. If available, a variable resistance or variable transformer may therefore be included in the circuit to facilitate control of the rate of heating. A heater can be operated satisfactorily, however, without the use of either of these devices. In this case, the exact concentration of the electrolyte for the desired rate of heating has to be determined empirically.

On this basis a heating unit was assembled as follows:



Two spectroscopic carbon rods, *a*, of 5-mm. diameter and 150-mm. length are immersed in the bath and rigidly fixed at a distance of approximately 10 mm. by means of a glass holder, *b*, as shown in the figure.

The holder is made by bending a glass rod of 3-mm. diameter into proper shape. The carbon rods are passed through glass tubes which are inserted into the cover, *c*, of the water bath. The leads of the 110-volt alternating current line are forced to the carbon rods by means of spiral springs, *d*.

Approximately 0.01 *N* sodium chloride solution is used as the electrolyte solution.

The water bath itself is a closed system, provided with a reflux condenser.

In an apparatus for the analysis of butadiene (*1*) a heating unit of the above description has been substituted and in use for several months. The main advantages are its small space requirement and the fact that it can be assembled in little time and without the use of critical materials.

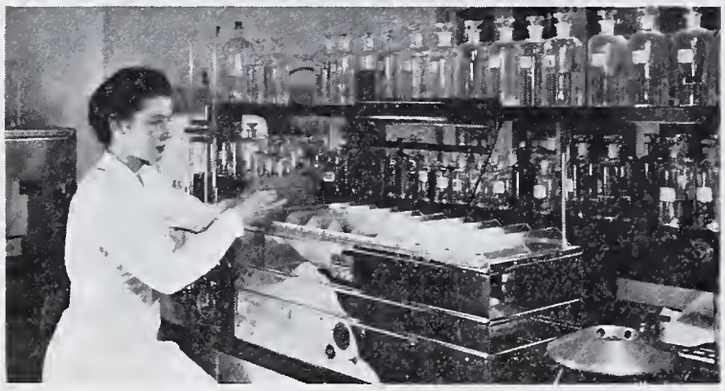
Literature Cited

(1) Tropsch, H., and Mattox, W. J., Ind. Eng. Chem., Anal. Ed., 6, 104 (1934).

NEW LABORATORY EQUIPMENT

Plastic Cover

Plastics has replaced another strategic metal, not only for the duration but also for the postwar period. A gable-type cover, devised for the U. S. Army Medical Corps serological bath, has the advantage over the former copper cover in being transparent as well as resistant to corrosion, and having a low coefficient of heat transfer which makes it an excellent thermal insulator to assist in maintaining constant temperature inside the water bath.



When these covers were put to use, moisture condensed on the inside, reached the saturation point, then abruptly ran down to the lower edges of the covers and dropped off into the water bath, instead of onto the test tubes, as with metal covers.

The equipment is being used by the Army for its Wassermann and Kahn tests with blood samples incubated at constant temperatures of 37° and 56° C. Molded of polystyrene, the cover was developed by the Precision Scientific Co., 1750 North Springfield Ave., Chicago 47, Ill.

Preliminary Test Bomb

A test bomb has been developed by the American Instrument Co., Silver Spring, Md., for making preliminary tests of chemical reactions at high pressures.

As normally supplied, the pressure seal is made by a copper gasket, totally enclosed for safety, between the head and the mouth of the bomb. Screwed into a standard Aminco superpressure opening for 0.25-inch outside diameter tubing in the head is a needle valve with side outlet for the same type of construction.

No opening is normally supplied in the bottom of the bomb. Where it is desired to maintain flow within the chamber, the bomb can be supplied with a standard connection, with plug to seal the bottom opening when not in use.

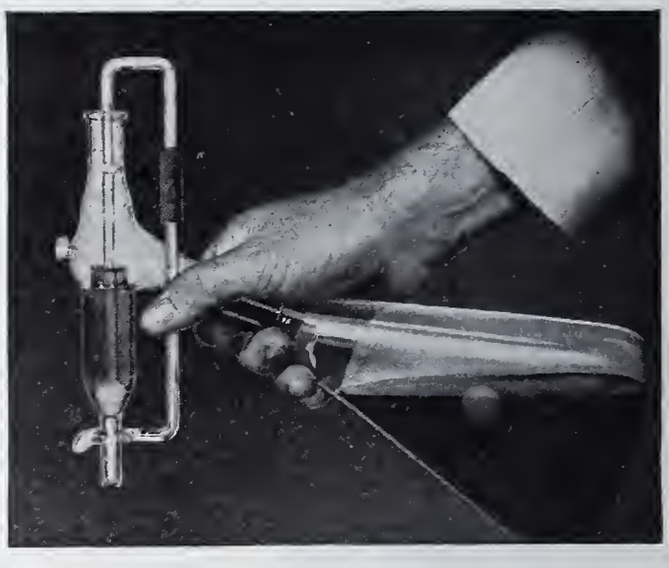
Standard construction is of high-strength steel. Each bomb is tested individually to 15,000 pounds per square inch and is intended for safe operation at a maximum internal pressure of 10,000 pounds per square inch. Calculated bursting pressure is over 40,000 pounds per square inch. The valve in the head permits charging at pressures as high as 5000 pounds per square inch and will seal the contents of the bomb up to the full working pressure of 10,000 pounds per square inch.

Water Testing Equipment

A test set for boiler water control analyses is announced by W. H. and L. D. Betz, Gillingham and Worth Sts., Frankford, Philadelphia, Penna. The set includes all the necessary apparatus and chemicals for the determination of hardness, alkalinity, chloride, sulfite, and phosphate. A special cabinet designed for use on table or wall holds apparatus and chemicals in a secure position and ready for instant use. A portion of the opened cabinet door forms a convenient acid-resistant laboratory work table and a fluorescent light provides correct illumination for the tests.

Automatic Pipet

In the automatic pipet illustrated, the measuring chamber is filled (slightly to overflowing) by tilting the flask to pouring position. When the flask is returned to vertical, the surplus drains back, leaving the chamber with an exactly predetermined volume. Its content is



let into the beaker by raising the plunger-lever with the thumb or by engaging the ring-hook with the far rim of the beaker and pressing downwards. Dispenser assemblies may be had for pipetted quantities from 5 to 100 cc., each dispenser operating with flasks holding 250, 500, 1000, 1500, or 2000 cc. Special combinations are also available. The pipet, catalog No. 850, is made by the Macalaster Bicknell Co., New Haven, Conn., or Cambridge, Mass

Technique of Assaying Amino Acids

The growing recognition of the importance of the amino acids as nutritional factors has raised the problem of exact assays, comparable to those now applied to vitamins and other such substances in commercial demand.

A rapid and practical technique of assay has recently been worked out. After considerable research work, the laboratories of the Wm. T Thompson Co., 2727 Hyperion Ave., Los Angeles 27, Calif., are in position to supply microbiological assays of the following amino acid

l-(+)-Glutamic acid	*l*-(−)-Cystine	*l*-(−)-Tyrosin
dl-Leucine	*dl*-Threonine	*dl*-Serine
dl-Valine	*dl*-Isoleucine	*dl*-Phenylalai
l-(−)-Tryptophane	*dl*-Methionine	*l*-(+)-Argini

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